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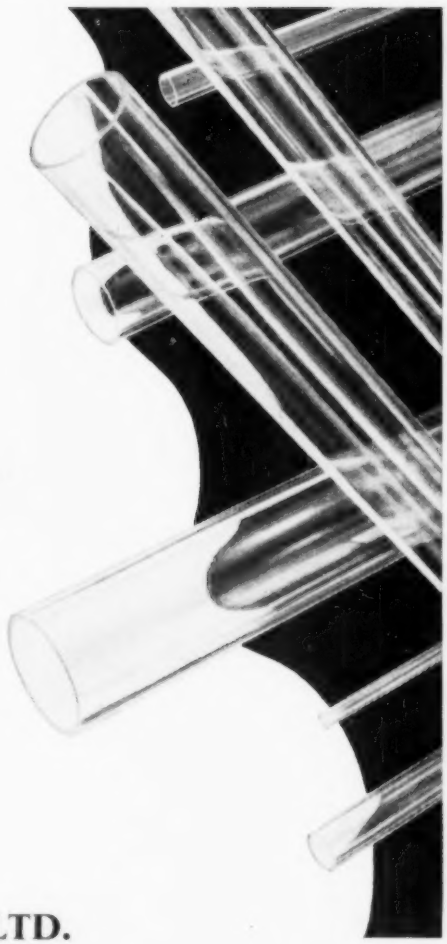

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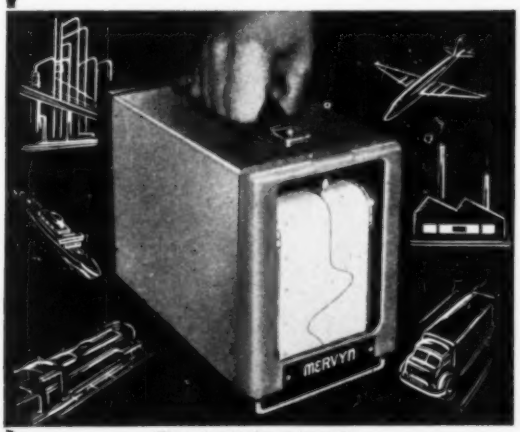

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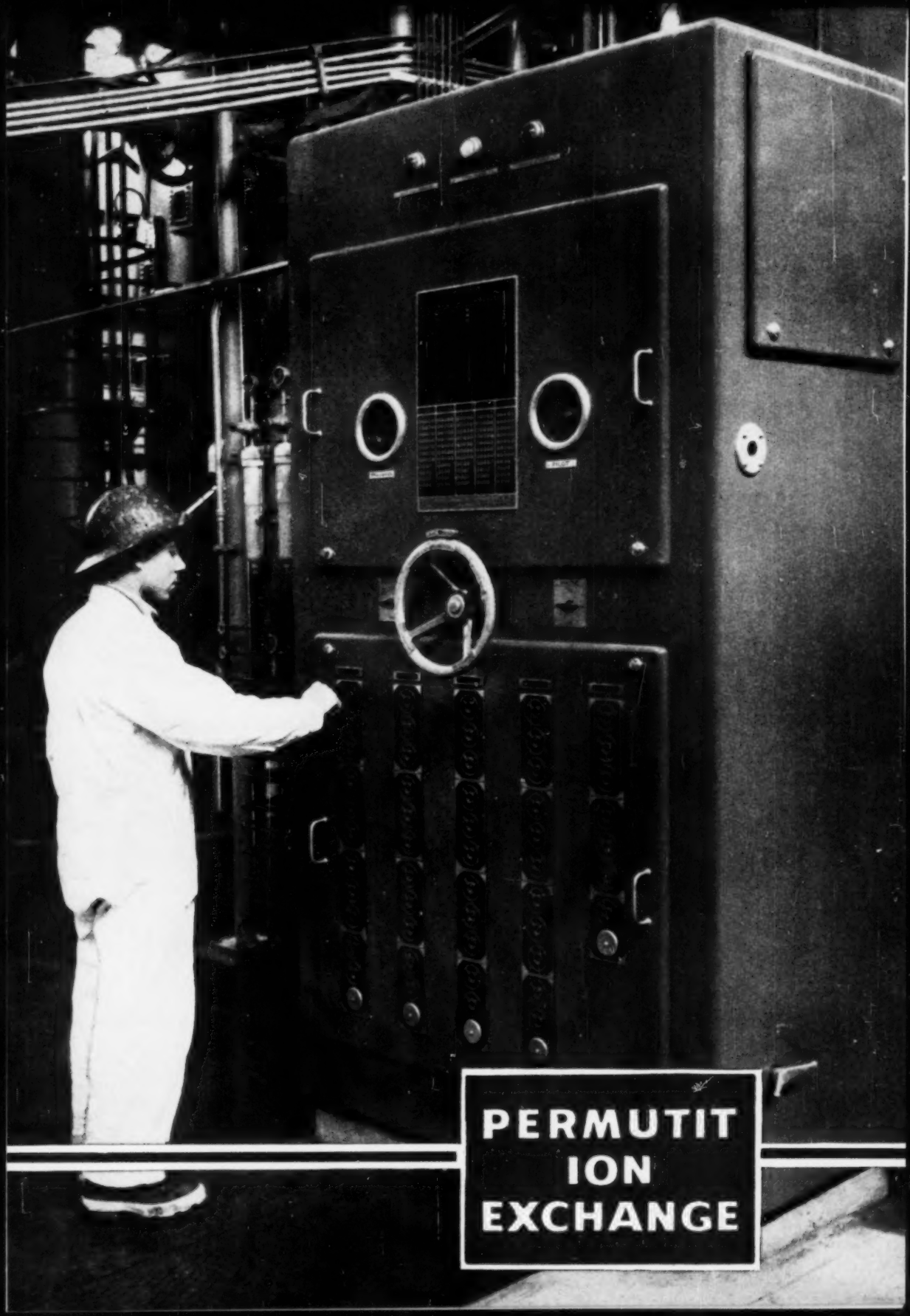
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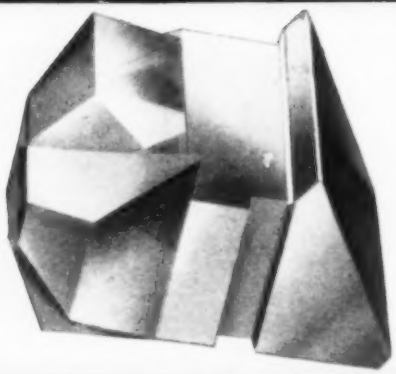
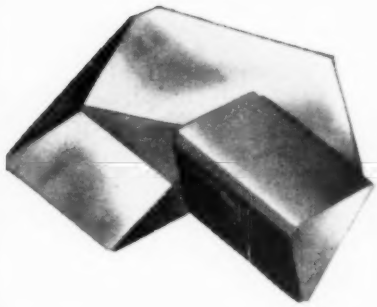

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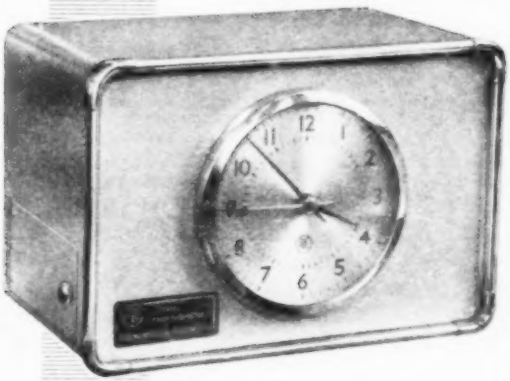

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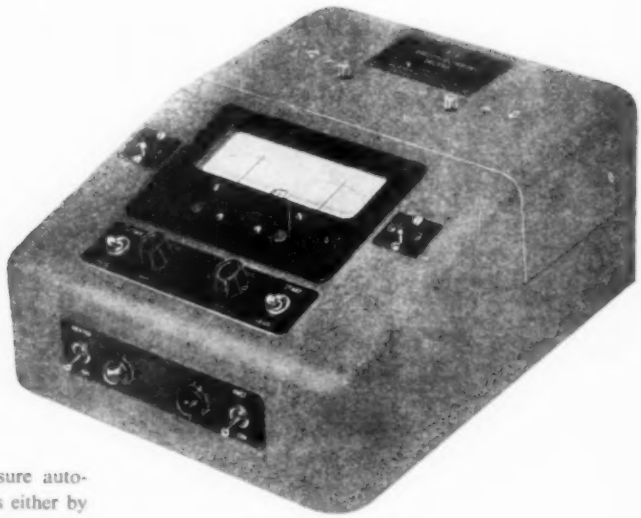

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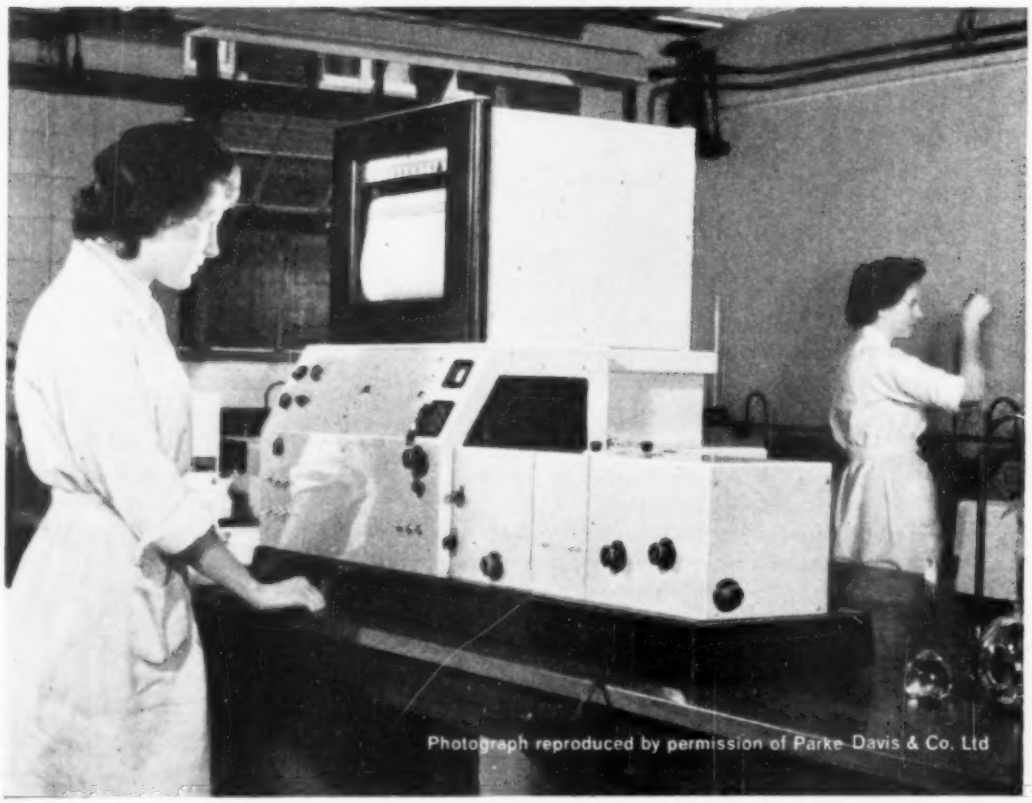

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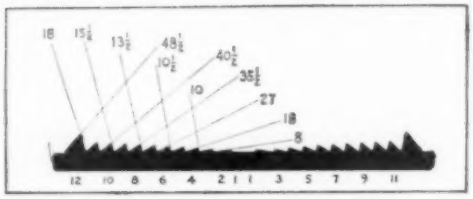

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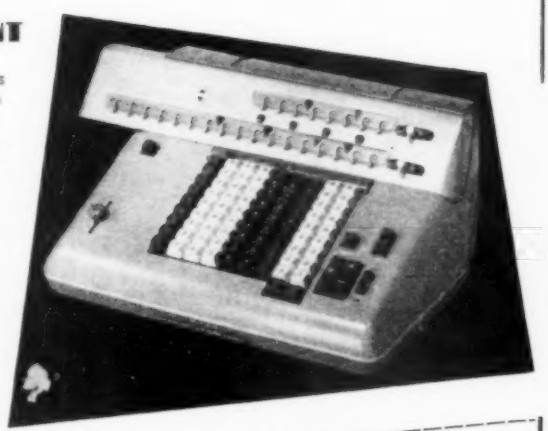

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A TIMELY REVOLUTION IN TECHNOLOGICAL EDUCATION

A SURVEY has been made to find out specifically what training a chemical engineer requires*. The results are interesting, since they provide a basis from which one may deduce a general principle regarding education of the technologist.

Chemical engineering is defined, in the *Encyclopædia Britannica*, as that branch of engineering which relates to the "design, construction, erection and operation of industrial plants in which matter undergoes a change of state or composition". The science, or craft, of the chemical engineer has been, and still is, the prime mover in developing the processes which serve to satisfy our technological needs. How best to fit the developing engineer for his task is, therefore, an important problem. The subjects which comprise his curriculum, as well as the time devoted to them, will determine, to a large extent, his ultimate effectiveness as a useful, well-integrated operator in industry.

With characteristic zeal for technological matters, the Americans, already in 1952, started to evaluate engineering training of all kinds†. An investigation was made by the American Society for Engineering Education. The method consisted in directing inquiries to a range of engineering colleges, industries and various professional organizations throughout the United States. The survey lasted three years: the replies were the considered opinions of authorities. Analysis revealed that, in a college engineering curriculum, 25 per cent of the time should be spent on mathematics and basic science, 50 per cent on engineering technology and the remainder on social studies, which include management techniques and applied economics. With its wide experience in industry and teaching, the Institution of Chemical Engineers in Britain recommends the following scheme: 45 per cent of the time to be spent on pure science and mathematics, 51 per cent on technological subjects and the remaining time on the social sciences, report writing and foreign languages.

No doubt these opinions are very useful, but they are nevertheless opinions. Realizing the need for factual information, Prof. R. E. Johnstone undertook an ingenious survey to find out in what way and to what extent the subjects taught at universities actually were being used by chemical engineers employed in British industry. With the aid of Mr. C. B. Lax, head of the Statistical Section at the Springfield Works of the United Kingdom Atomic Energy Authority, a questionnaire was so designed that replies would be "factual, quantitative, and amenable to statistical analysis". The questionnaires were sent to all corporate and graduate members of the Institution who were resident in the British Isles, except those engaged in full-time teaching. From a list of twenty-four sciences and techniques, engineers were requested to indicate which of these they had applied during the previous working week. To supplement information gained readily from almost two thousand engineers, Prof. Johnstone analysed his personal diary, a record of work done in industry over a period of twenty-eight years. This included most chemical engineering jobs in an eminently representative set of industries.

Results from these two complementary sources have been used, most elegantly, to design a suitable scheme of study for the engineer. Prof. Johnstone derived two limits within which the curriculum should lie. One limit, the "applied bias", represents a course of study in which the applied, technological type of subject predominates; the "pure bias", in contrast, stresses subjects for which there is not necessarily direct application. These limiting cases are summarized; so, too, is the scheme of the Institution as follows:

Subject	Per cent time of curriculum		
	Pure bias	Applied bias	Scheme of the Institute
Chemical engineering science	45	44	42
Pure science	22	14	34
Management and economics	6	19	2
Higher mathematics	13	9	11
Other engineering science	8	8	9
Report writing and foreign languages	6	6	2

Agreement with the scheme recommended by the Institution is remarkably close, except for pure science. Even if an engineering course with a leaning toward the 'non-applied' is required, the amount of time recommended for pure science by the Institution is rather excessive (34 per cent versus 22 per cent). At the other limit, namely, with an applied bias, more than twice as much time would be assigned to the study of pure science than is actually required by industry. There would be a consequent deficiency in time spent on training in management and economics.

Prof. Johnstone points out that much of the chemistry and physics used by practising chemical engineers is not above university entrance-level. Time allocated to such subjects probably could be spent far more effectively on topics of real engineering virtue. Moreover, as Prof. Johnstone states, "a scientist can practise his profession at a university, the engineer cannot". Engineering is a "large-scale activity which requires considerable resources in men, materials, accommodation and equipment before it can be carried on at all". Not only is a university engineering laboratory a very different

* *A Survey of Chemical Engineering Education and Practice*, presented by Prof. R. Edgeworth Johnstone at a meeting of the Institution of Chemical Engineers, January 24, 1961.

† *Report on Evaluation of Engineering Education*, 1952–1955. American Society for Engineering Education, Illinois.

place from an industrial factory, but also the work done is very different. Much of this information serves to confirm what experienced engineers have known for some time. Nevertheless, the advantages of such a survey are: "that it places the facts of the situation beyond reasonable argument" and "it provides quantitative data in place of judgments that have hitherto been largely qualitative", as Prof. Johnstone concludes. He and his colleague are indeed to be congratulated on their survey.

Now, the facts are plain. So, too, is the inescapable question: Would it not be of greater advantage to teach technology elsewhere rather than at a university?

There have been numerous complaints from industry that universities do not produce the kind of engineer required. From the academic side come weak rejoinders that industry does not know what is good for it. At last the solution is clear. Considering the demands of industries (and this survey has proved their demands most reasonable) the training of an engineer could probably be accomplished best by institutes of advanced technology, working in close liaison with industry. They would be similar in function to the practice-school of engineering at the Massachusetts Institute of Technology. Students there spend a large portion of their time in selected industries, but preserve close contact with the Institute.

The advantages are clear. Student engineers would have far more confidence when finally they are ready to begin their professional careers, due to their familiarity with actual processes of production. They will have learned, among a host of useful techniques to do working designs of fractionating towers with all the non-idealities involved, heat-balances on commercial furnaces, density measurements on fluid catalytic reactors and regenerators in operation, process 'trouble-shooting', and investigations on the idiosyncrasies of large-scale automation. They may have delved into marketing strategy, into the operation of consumer demand and/or company policy in determining the industrial processes to be used and into the methods of design for the best 'pay-out' on investment of capital. Moreover, they will gain an insight into the art of managing personnel and process operations. Along with this, they will develop an intuition for the enlightened guess-work which characterizes much of technology. Industry would welcome technologists who have been nurtured under its wing, so to speak.

Apart from the immeasurable financial advantage of having more efficient engineers in industry, the State would spend less in educating them. The unit cost of training a technologist is conceivably far less at a technical institute than at a university. Since industry will be supplying the *ne plus ultra* of engineering equipment, no longer will universities have to budget for expensive, though inadequate, substitutes. No longer will the academic staff suffer the malaise of not knowing whether to emphasize the 'applied' or the 'pure' aspects of learning. Happily, they will be free to encourage disinterested inquiry, the real function of a university.

If it seems better to teach technology in institutes separate from universities, then the question arises whether the engineering research which is carried out by universities should rather be done elsewhere. It is well to examine how the engineer aids technology in his research. In general, chemical engineering research appears as a stage after pure chemical research. When a new compound or a new way of synthesizing or converting known compounds has been discovered by a research chemist, and a market demand is established, the research engineer takes up the tale. He has to specify ultimately the type, size and operating conditions of equipment to yield the products required. Bench-scale experiments or pilot-plant work is used. Although pilot-plants are very expensive, it is often safer to base commercial designs on them, since scale-up from small experiments tends to be risky.

The theoretical problems involved are often quite abstruse. For example, the kinetics of the reacting system is usually a far cry from ordinary chemical kinetics, yielded by test-tube experiments. Complexities arising from heat, mass and momentum transfer must be taken into consideration. Engineering systems are non-ideal and hence not readily amenable to formulation. But here is where engineering research has displayed its particular genius in setting up mathematical models, often rough, but which serve, nevertheless, as reasonable approximations in describing physical systems. However, as the research engineer delves deeper into the theoretical aspects of these problems, he finds that he needs the tools of the applied mathematician and chemical physicist more and more. He needs to command a knowledge of mathematical probability theory, stochastic processes, variational principles, the absolute differential calculus, statistical mechanics and perhaps topology as well, to probe effectively into physical problems. Formal engineering education tends to fit him rather inadequately for this.

To do advanced experimental engineering research is a very costly business; consider items like shock-tubes, equipment for investigating turbulent shear flow, electronic computers, the apparatus for study of plasma-jets and so forth. Not only does industry have a personal commercial incentive to do such work, but also it commands the resources. It is unjust to say that industry is concerned entirely with empirical research. Consider, for example, from whence hails the revolutionary theory which explains the effect of interfacial turbulence on mass transfer. Large-scale industry is sophisticated enough to appreciate that to have research workers with advanced theoretical background in physics, chemistry and applied mathematics doing fundamental research can lead to unexpected results with vast technological implications.

Bearing all this in mind, it seems very obvious indeed that not only technology but also research in technology are out of place in the universities. Thus, the following system is recommended: technical institutes and industry will co-operate in producing efficient technologists; universities will play their part in educating first-rate applied mathe-

maticians and chemical physicists to do research work in industry.

Sir John Cockcroft has stressed, on several occasions, the need for institutes of technology quite separate from universities proper. This timely transfer of technologies from the domain of the university is also contemplated by Dr. S. C. Curran in his article on p. 128 of this issue of *Nature*. Like winged *Drosophila*, they will take off to flourish in a new environment, leaving their erstwhile neighbours in the university to breathe more freely, too. The function of an institute of technology must not be confounded with that of a university. To preserve efficiency in this increasingly competitive world we must follow inevitably the economic dictum of 'division of endeavour'.

The ideal division thus seems to be with universities on one side. They will stress sound instruction and lecturing of the highest quality in the 'pure' fields. Research at the university should be of secondary importance and more of a fundamental, theoretical kind. The tenor of 'the academic marketplace', it is hoped, will disappear. Academic staff will no longer be compelled to 'publish or perish'. On the other side, there will be the institutes of technology, well integrated with industry, which will thus exert a greater influence in determining the educational background of its technological force. Industry should not be denied this privilege. After all, it does supply the purpose for training technologists. The traditional, though lately diminishing, sovereignty of the universities should remain intact.

The net effect will be a stream-lining of physical science and a more potent research force. Technologists will be the fully fledged empiricists whom industry requires and they will know precisely what technological questions to ask. In the research departments of industry there will be a complementary force of well-equipped research workers. They will know where to seek the answers. Altogether, it seems reasonable that this would be a far more efficient, more manageable, neater and more powerful system than to have semi-empiricists of the twilight zone who neither know exactly what questions to ask nor how to set about answering them.

THE WORKS OF PAUL EHRENFEST

Collected Scientific Papers

By Paul Ehrenfest. Edited by Martin J. Klein. Pp. xii+632. (Amsterdam: North-Holland Publishing Company, 1959.) 90*s*.

PAUL EHRENFEST, the Dutch theoretical physicist, was born in 1880, took his doctor's degree under Boltzmann in 1904, and succeeded H. A. Lorentz in the chair of theoretical physics at Leyden in 1912; he remained there until his death in 1933. His active life therefore spanned most of the great period of transition from classical to modern theoretical physics, and his collected papers show the important part he had in helping on, interpreting, and clarifying this transition.

The earlier papers are mainly on classical statistical mechanics; in particular, Ehrenfest was much concerned with elucidating the basic ideas of Boltzmann's theory. To the same period belongs the great article on the fundamentals of statistical mechanics which was written in collaboration with his wife when he was in Leningrad, and was published in the *Encyclopädie der Mathematischen Wissenschaften* in 1912. In the later papers he dealt mainly with quantum statistics, methods of quantization and related topics. In particular, his famous paper on adiabatic invariants, which had so considerable an influence on the development of quantum theory, is also an outcome of his work on statistical mechanics.

His inaugural discourse at Leyden in 1913 is a good example of some characteristic features of his work, although it is only a semi-popular exposition. His subject was the downfall of the theories of the æther. He pictures an observer situated at the centre of a vast hollow reflecting sphere. The observer has by him a powerful lamp which he uses to send out a short flash of light uniformly in all directions. A little later the observer will see the whole inner surface of the sphere lit up simultaneously. Next, Ehrenfest asks us to think of the same vast sphere (and the observer) moving uniformly. If Newton were the observer, he would again expect to see the whole inner surface lit up simultaneously. Fresnel, however, would expect to see just a narrow band of illumination on an equator of the sphere. The illuminated band would then rapidly broaden until it reached the poles. Finally, Einstein, with the help of Lorentz, would expect to see the same as Newton. Here we have the elegant model used to illustrate a physical idea and to bring out the essential features of the problem. The discourse also provides an example of Ehrenfest's constant concern with the careful discussion of basic principles.

The short paper on the rotating dipole—written in collaboration with G. Breit in 1922—illustrates very well the difficulties of the period up to 1926 during which Ehrenfest was striving to clarify the old quantum theory of Bohr. It was a time when physicists stuck very closely, and very literally, to classical concepts. Ehrenfest and Breit of course had a model. It was an electric dipole which could rotate freely about an axis which was perpendicular to the dipole and passed through its mid-point. When the angle of rotation became $\pm f.2\pi$ where f is any large number (rational or irrational) the dipole is thrown back elastically. It is perhaps not surprising that Bohr's quantization rules could not cope with this system in a reasonable way. (It has a somewhat curious period and a quasi-period). However, Ehrenfest and Breit showed, in an elegant analysis, that the correspondence principle could be used to make the quantization rules yield the correct value for the angular momentum.

Ehrenfest admired H. A. Lorentz and held him in great respect; his obituary notice for Lorentz is included here. However, it is in an appreciation of Lorentz as a research physicist that we see his celebrated sense of fun for once managing to get into his writing. This appreciation was written on the occasion of Lorentz's seventieth birthday in 1923. The third paragraph is as follows:

"Perhaps too our ear suddenly recalls the variety of nuances of sound with which we have heard the name Lorentz pronounced by brilliant men of all nations. If an American pronounces it, or a

Frenchman or a Russian or a Scandinavian, or Planck, Boltzmann or Einstein, one can surely notice that each one means his own Lorentz. If a Netherlander has to mention the name Lorentz, he does it with pride, but softly, as if he fears that 'Perhaps Mr. Lorentz will be able to hear that he is being spoken about'." J. Hamilton

GENERAL PLANT CELL PHYSIOLOGY

Plant Physiology

A Treatise. Edited by F. C. Steward. Vol. 1A: Cellular Organization and Respiration. Pp. xxvii+331. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 13 dollars.

THIS is the first volume in order of numbering of the treatise on plant physiology in process of production under the general editorship of Prof. F. C. Steward, although it is actually the second to appear, the volume numbered 2 having already been noticed in these pages. Volume 1A contains three articles, the first on the plant cell and its inclusions, the others on some aspects of general plant cell physiology. It is desirable, indeed necessary, that a treatise on plant physiology should begin with an account of the cell, the system in which physiological actions take place, and R. Brown gives an account of this which is generally adequate and up to date. The conclusions drawn during recent years regarding cellular membranes, largely as a result of electron microscopy, are properly stressed, but as in all probability comparatively few readers will have had actual first-hand experience of the electron microscope, a brief description of how results are obtained by its use, and the reasons for the accepted interpretation of the structures so observed, might not have been out of place. These conclusions, it may be noted, in so far as they refer to the plasmatic membranes, fall well into line with those suggested by the conception of free space due to Briggs and Robertson.

The second article, by B. Vennesland, is entitled "Proteins, Enzymes, and the Mechanism of Enzyme Action". There is reason to believe that protoplasmic proteins, at least in many cells, are largely held in enzymes, and this account deals mainly with enzyme structure, which includes the structure of protein molecules, and the mechanism of enzyme actions. No classification of enzymes has ever received universal acceptance, and the author does not attempt a new one. However, in dealing with enzyme reaction mechanisms three different kinds of enzymes are considered separately. These are: (1) oxidation-reduction enzymes containing firmly bound prosthetic groups; (2) enzymes containing thiamine or pyridoxine derivatives; (3) pyridine nucleotide-linked dehydrogenases. Other aspects of enzyme reactions considered are their kinetics, inhibitors, group transfers and the problem of the active site. The article provides a good and succinct account of the present position of our knowledge of enzyme structure and action.

The final article deals mainly with what is generally called the mechanism of respiration and gives satisfactory accounts of the various ways by which it is now supposed the respiratory substrates are broken down, though perhaps more attention might have been given to the action of lactic dehydrogenase leading to the formation of lactic acid. I cannot agree with the statement that the production of lactic acid from pyruvic acid is "relatively rare in higher plants, though traces of lactic acid are found in potato fermentation . . . and in wheat and rice". Actually the production of lactic acid has been observed in other plants, while in potatoes Barker found that at the beginning of a period of anaerobiosis the production of alcohol and carbon dioxide might be negligible in comparison with the production of lactic acid. In tomato stems a production of lactic acid recorded as amounting to 13·7 per cent of the carbon dioxide produced can scarcely be described as a trace. Again, I do not think that the acceptance of the universality of the EMP pathway was as surprising and uncritical as the authors suggest. And, was such a universality as dogmatically asserted as they say? Surely it was no more than a tacit acceptance, a view which was readily modified when definite evidence of an alternative pathway was forthcoming.

The production of the volumes of this treatise is of the high quality we have come to associate with the publications of the Academic Press.

Walter Stiles

BONDS, BANDS AND SEMICONDUCTORS

Progress in Semiconductors

Vol. 5. Edited by Dr. Alan F. Gibson, Prof. R. E. Burgess and Dr. F. A. Kröger. Pp. vii+316. (London: Heywood and Co., Ltd., 1960.) 63*s*. net.

IS the band theory of semiconductors the modern phlogiston theory? Lavoisier's discovery of oxygen brought about the downfall of the latter; by some eyes the discovery of vitreous and liquid semiconductors is seen as the herald of the fall of band theory. Others, less extreme, see a better historical analogy to the current band-bond battle in the wave-particle war and consequently expect an amalgamation of the two theories. Band theory has reached a high degree of sophistication, as is evidenced by most articles in the latest addition to this invaluable series of progress reports. The chemical bond approach discussed by E. Mooser and W. B. Pearson is by comparison in its infancy. The authors discuss rules derived from it for predicting semiconductivity (their own rule appears in a modified form which allows for cation-cation bonds), and then attempt to classify semiconductors on the basis of their structures. In the concluding sections some intriguing empirical graphs relating semiconductor and structural parameters are reproduced. Some discussion of alloy semiconductors, especially as regards superlattice formation, might have been included.

Optical properties, as the editors admit, predominate in this issue. T. P. McLean reviews recent work on the absorption edge spectrum of semiconductors, in particular of germanium and silicon. B. Lax and S. Zwerdling have obviously striven to minimize the mathematics in their survey of magneto-optical phenomena. In the well-balanced meal they produce there is both meat for the specialist and gravy for the beginner. T. S. Moss, in a short

article on indium antimonide, omits chemical and structural work in his haste to reach the optical properties where, of course, his interests lie. The sixty-nine references might well have been considerably increased. His final section on applications is of special interest.

The electrical properties of semiconductor surfaces, a very difficult subject, is ably reviewed by T. B. Watkins, with 26 figures in the text and 99 references. He concludes with some pertinent comments on the effects of surface conditions in semiconductor devices.

J. Appel's article on thermal conductivity of semiconductors is concerned almost exclusively with the theoretical aspects of the subject, which are developed from two angles: first, phenomenological, and secondly, quantum mechanical. The conclusion touches very briefly on the practical problem of thermoelectric cooling. The author is to be congratulated on the clarity of his exposition since English is not his native tongue.

R. R. Haering and S. Mrozowski give a masterly survey of the band structure and electronic properties of graphite crystals. The authors divide the properties they discuss into two groups: those which can be explained on a band model and those which cannot as yet. This article should inspire further work as it stresses unsolved problems.

A welcome addition at the end of the book is a table of contents of past and future volumes. Many interesting articles on the properties of semiconductors in general and the elements germanium, silicon, carbon, tellurium and bismuth in particular are in preparation. There are, however, several bad gaps in the programme. Compound semiconductors and their alloys seem ignored entirely. Interest in these stems especially from thermoelectric cooling and thermoelectric energy conversion, and so it is not surprising to find these topics absent too. No proposed article deals specifically with any topic in semiconductor chemistry or crystallography. This may be deliberate policy, but, if so, perhaps the editors should change the title of the series to *Progress in Semiconductor Physics*.

B. R. Pamplin

MICROSCOPIC STUDIES OF REFLECTING SURFACES

Surface Microtopography

By Prof. S. Tolansky. Pp. viii+296. (London: Longmans, Green and Co., Ltd., 1960.) 55*s*. net.

PROF. TOLANSKY describes here a series of investigations made with the collaboration of his research students over the past fifteen years or so. Most of the work has involved the use of a microscope, in some cases with phase-contrast techniques, but more often with the multiple-beam interference methods for which Prof. Tolansky is famous. The space devoted to each topic is necessarily short, but each is illustrated by many beautiful photographs printed on the glossy paper used throughout the book.

As the author remarks in the preface, the book is a kind of atlas, in which the 359 excellent pictures show 'maps' of various physical features of the structure of solid surfaces and their behaviour under the action of physical agencies. It is remarkable how wide a field of study is open to the application of multiple-beam interferometry in spite of the rather stringent limitations set by the need for the specimen to be able to withstand the cleaning and vacuum evaporation of silver without change. A large part of the book is concerned with crystals, particularly diamond, and the investigations cover aspects of their growth, cleavage, and etching, as well as studies of hardness and wear in relation to the crystalline structure. Other sections of the book include selected features of work on thin films, metal crystals and hardness testing, quartz crystals, silicon carbide, mica, and piezoelectric oscillators. The methods used are capable of extremely high sensitivity, and it is claimed that, in favourable cases, height differences of only a few angstroms can be estimated.

Something of value can be found here by those engaged in any of the branches of study included in the book, but in addition the book as a whole is valuable to students of optical techniques and to anyone who is likely to be inspired and stimulated by a fascinating account of highly informative experimental work carried out with very simple means. The text conveys the enthusiasm Prof. Tolansky so obviously has for this subject, and the way in which the story of each investigation is related holds the imagination and carries the reader along from the unravelling of one knot in the mystery to the next.

The first five chapters are concerned with the basic techniques of multiple-beam interferometry, and include better illustrations and diagrams of the equipment than have been published before. The diagrams, however, are still barely adequate to give more than just an idea of the arrangement of the apparatus required, and in some respects are actually misleading. One or two near-scale drawings of the apparatus would have been much more helpful to anyone wishing to try the technique, although this book is obviously not intended to be an instruction manual.

The remaining eighteen chapters are arranged in six groups, covering the topics already mentioned, and accompanied, where necessary, by further description of special techniques. The volume is concluded by two addenda introducing two more special interference systems, and two bibliographies confined to the publications of Prof. Tolansky and his students, and to the theses of his students. Other references are given in the text of the book as they occur. Some misprints and distortions of language seem to have escaped the proof-reading and editing, and this is annoying in a book of this price and quality; in a few cases the account of an experiment seems to have been shortened to an extent which makes the argument difficult to follow. A possible case of this occurs on p. 194, concerning the electro-deposition of layers, where the explanation seems to show an inconsistency with the illustrations. The point at issue here would best be resolved by line diagrams of the profiles of the edges of the layers, as in Plate 242. More profile diagrams of this kind would be helpful at other places too.

These minor defects do not detract much from the value of the book, if at all, as its chief function would seem to be as an introduction to the unique studies described more fully in the papers and theses on which it is based, and as a fascinating and stimulating account of a great deal of masterly experimental work.

J. W. Gates

Gasdynamic Discontinuities

By Wallace D. Hayes. (Princeton Aeronautical Paperbacks, No. 3.) Pp. v+68. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 1.45 dollars; 11*s*. 6*d*. net.

Small Perturbation Theory

By W. R. Sears. (Princeton Aeronautical Paperbacks, No. 4.) Pp. v+63. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 1.45 dollars; 11*s*. 6*d*. net.

Higher Approximations in Aerodynamic Theory

By M. J. Lighthill. (Princeton Aeronautical Paperbacks, No. 5.) Pp. v+147. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 1.95 dollars; 16*s*. net.

High Speed Wing Theory

By Robert T. Jones and Doris Cohen. (Princeton Aeronautical Paperbacks, No. 6.) Pp. v+243. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 2.95 dollars; 24*s*. net.

THAT there is a strong case for publishing 'The Sonnets' independently of the 'Complete Works' is scarcely to be disputed. There is at least as strong a case for separate publication of some, if not all, of the parts of the heterogeneous and overlapping collection of papers by dozens of authors which will eventually make up the twelve volumes of the Princeton Series on "High Speed Aerodynamics and Jet Propulsion". From the pleasantly rounded presentation of the individual contributions it appears that the ties of co-operative endeavour did not too severely constrict the individual authors. Consequently, the six paper-back extracts so far republished should meet a lively demand from those who have been attracted by the two-inch-thick volumes of the original series and from those who have been daunted by them; they will be warmly welcomed by students.

Numbers 1 and 2 in the paper-back series are concerned with rockets. Numbers 3, 4, 5 and 6 are respectively Vol. 3 Section *D*, Vol. 6 Section *C*, Vol. 6 Section *E* and Vol. 7 Section *A* of the Princeton Series, all widely applauded when they first appeared a few years ago. Number 6, by Mr. and Mrs. Jones, stands particularly well on its own as a really excellent text-book. In subsequent paper-backs in this series the publishers ought briefly to explain their system of cross-references.

Beiträge zür Physik und Chemie des 20 Jahrhunderts:

Lise Meitner, Otto Hahn, Max von Laue zum 80 Geburtstag. Herausgegeben von O. R. Frisch, F. A. Paneth, F. Laves und P. Roabaud. Pp. xii+285+3 tafeln. (Braunschweig: Friedr. Vieweg und Sohn, 1959.) 29.50 D.M.

THIS is a collection of essays which was published to mark the eightieth birthday of three distinguished scientists, Lise Meitner, Otto Hahn and Max von Laue. About two-thirds of the book is in German and the remainder is in French and English. Some of the essays are historical and others contain accounts of very recent work; all are designed to demonstrate the importance of the early fundamental work of the three great pioneers. Many of the papers undoubtedly make extremely important contributions to knowledge; the present writer is much more concerned, however, with another aspect. Prof. Hooykaas of Amsterdam, speaking at the "Technology and Purpose" Conference at Oxford in 1959, deplored the impersonal nature of modern scientific writing. He pointed out as an example that in early chemical text-books the experimenter was portrayed in full academic robes whereas in modern texts the experimenter is eliminated; the test-tubes apparently support themselves.

The inestimable value of this collection of essays is that it begins to sketch in the character and personality not only of the scientists honoured but also of the many contributors. For example, we find Prof. Przibram recounting anecdotes about other learned professors, we find Dame Kathleen Lonsdale throwing stones into a pond and we find a paper by Prof. Rosenblum on "Spectres Magnetique des Particles Alpha" beginning "Je regarde mon pouce . . . ".

I can read German only with great difficulty and continual recourse to a dictionary; but in spite of this three vivid impressions have been obtained. The first is of the explosive growth of science in the fifty or so years covered; the second is of the immense fruitfulness of the work of the three great pioneers which stimulated this growth; the third is that perhaps the greatest tribute of all is simply that they should inspire the contributors to write in such warm human terms on subjects that would normally be regarded as completely objective and impersonal.

C. A. Taylor

Fonctions Sphériques de Legendre et Fonctions Sphéroidales

Tome 3. Par Dr. Louis Robin. (Collection Technique et Scientifique du Centre National d'Études des Télécommunications.) Pp. viii+289. (Paris: Gauthier-Villars, 1959.) n.p.

THIS is the concluding volume of Dr. Robin's encyclopædic treatise on Legendre functions and related topics; for the English reader, it supplements Hobson's 1931 treatise by its account of work done in recent years. The main sections deal with addition theorems, zeros, problems relating to a spheroid, torus or cone, and with functions allied to the Legendre functions, such as the Gegenbauer polynomials and functions. There is also a section on the spheroidal functions, mainly an epitome of the full account in Meixner and Schäfke, *Mathieusche Funktionen und Sphäroidfunktionen*, and a brief note on spherical Bessel functions. There are numerous bibliographical references, and useful lists of available numerical tables. The text, with its inevitable mass of complicated formulæ, is scarcely light reading, but the book forms a most valuable work of reference, well deserving a place in any mathematical library.

Biochemical Preparations

Vol. 7. Edited by Henry A. Lardy. Pp. ix+102. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1960.) 42*s*. net.

THE series of *Biochemical Preparations* needs no special commendation in these columns. It will be sufficient to say that Volume 7 extends and enhances the reputation of established usefulness and merit which the series has already gained, and that it betrays no sense of strain or dilettantism in the choice of matter forming the list of contents.

Of particular interest in the present volume are the methods for the preparation of N-acetylneuraminic acid from human milk; for preparing phosphorus-32-labelled adenosine di- and triphosphates

by both chemical and enzymic means—the latter neatly adapted to give A-P-P*-P and A-P-P-P* as well as A-P-P*-P* ; and for a preparation of flavine adenine dinucleotide from the mycelium of *Eremothecium ashbyii* which gives better yields than the more usual preparations from animal tissues or yeast. The other methods included are for : β-amino*iso*butyric acid ; carbamyl phosphate ; (−)-carnitine chloride ; cerebrosides ; coproporphyrin III tetramethyl ester ; 6-deoxy-6-fluoro-D-glucose ; dihydroxyacetone phosphate ; D-fructose 1-phosphate ; 3-hydroxy-L-kynurenine ; hydroxypyruvic acid phosphate ; methylmalonic semialdehyde ; potassium dihydrogen $L_s(+)$-*iso*citrate ; S-palmityl coenzyme-A ; stearic acid, methyl stearate and homologous compounds ; stigmasterol ; and tetrahydrofolic acid.

This simple enumeration will be sufficient proof of the pertinence to the needs of the research worker which the volume maintains. Since a different editor-in-chief is appointed for each volume of *Biochemical Preparations* it is fitting to record here our thanks to Prof. Lardy, who has so successfully compiled and presented the volume under review.

F. H. MALPRESS

Hospital Infection

Causes and Prevention. By R. E. O. Williams, R. Blowers, L. P. Garrod and R. A. Shooter. Pp. x+307. (London : Lloyd-Luke (Medical Books), Ltd., 1960.) 35*s*. net.

A SYSTEMATIC approach to the causes and prevention of hospital infection is much to be welcomed. Accurate records are meagre and the problem is one which belongs to everybody and, consequently, to no one. This new book by four well-known specialists can fairly claim to provide a comprehensive review of how infections are spread, how they can be controlled and, more apposite, how they can be prevented.

Since streptococcal infections now cause no real difficulties—they still respond to penicillin and have acquired no resistance to the drug—the book is mainly concerned with the staphylococcal infections which, because of their resistant strains, are the main source of infection and worry in hospitals today. For a medical publication, the cautious and almost agnostic approach is refreshing—no views are put forward unless they can be substantiated with demonstrable facts. The text is clear and logically presented, and adds to the value of a book which should be useful not only to pathologists and bacteriologists but also to surgeons, pædiatricians, sister tutors, hospital administrators, and equally important, hospital architects.

KATHLEEN M. HAWKINS

An Introduction to Statistical Thermodynamics

By Terrell L. Hill. (Addison-Wesley Series in Chemistry.) Pp. xiv+508. (Reading, Mass., and London : Addison-Wesley Publishing Company, 1960.) 74*s*.

THIS introductory text on equilibrium statistical thermodynamics is based on the elementary principles of quantum mechanics and covers a wide field of applications in both chemistry and physics. Classical statistics are not developed independently, but there is a short chapter on the transition from quantum statistics in which the treatment, however, is so curtailed as to be of doubtful value to the uninitiated. Indeed, because of the extensive coverage of modern developments, it has been necessary throughout the book to condense the mathematics in some measure, and the student who is specializing in either chemistry or physics might feel that the inclusion of much material, which inevitably must be outside the scope of his course, does not compensate for this lack of mathematical detail.

The book is divided into four parts, the first of which is devoted to a discussion of the various ensembles and to the statistical-mechanical treatment of the second and third laws of thermodynamics. The fundamentals having thus been established, their applications to systems of independent particles are next considered, and in the third part, which is concerned with interacting particles, there appears a comprehensive survey of the problems encountered in the study of liquids and solutions. Finally, distributions such as those of Fermi–Dirac and Bose–Einstein are derived.

Misleading statements noted include a reference to the temperature of a single molecule (p. 71) and an implication that the dimensions of both an area and a volume in classical phase-space are the same, namely those of Planck's constant (p. 113).

H. STEEPLE

British Scientific and Technical Books, 1953–57

Edited by L. J. Anthony. Pp. ix+251. (London : James Clarke and Co., Ltd., 1960. Published for Aslib.) 56*s*. net.

THIS volume is essentially a continuation of *British Scientific and Technical Books, 1935–1952*, following the pattern of that earlier bibliography and including entries for the more important and useful books on science and technology published in the British Commonwealth between January 1953 and December 1957. Entries are based on those in the *Aslib Book List* and relate primarily to books published through normal trade channels by commercial publishers. Government publications, monographs, and reports and papers issued by societies and institutions have been included only if they represent important contributions to the knowledge of the subject not otherwise obtainable. Entries for later editions of books noted in the earlier volume are included only if the new edition contains significant alterations or additions ; straightforward reprints are omitted. Besides including those books in such fields as documentation, libraries, psychology, planning and architecture which are of interest to technical readers, every effort has been made to include in the scientific and technical sections books which assist the understanding of the subject by non-technical readers.

The subject headings in the classified bibliography follow the order of the Universal Decimal Classification, but some modifications which have come into use since the issue of the latest Abridged English Edition have been used. Under each class heading entries are arranged alphabetically by author and the entries are annotated to indicate their scope and suitability for three different classes of readers or whether they are reference works such as dictionaries, encyclopædias, etc. There are an author index and a subject index. The volume is admirably produced, but its value would be appreciably enhanced if the time lag between the date of the latest entries and that of publication of the volume could be reduced ; on the present basis of compilation it does not seem too much to expect that this interval could be reduced by at least a year.

R. BRIGHTMAN

PROPOSED MOLECULAR MODELS OF GRAMICIDIN S AND OTHER POLYPEPTIDES

By Dr. DONALD T. WARNER
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Introduction

ONE of the most interesting problems in protein and polypeptide chemistry is the tertiary structure or conformation of the amino-acid chain and the relation of this structure to the biological activity of the compound. Some of the probable forces holding the polypeptide chain units in position have been studied extensively; but since the initial work of Pauling *et al.*[1] the hydrogen-bonding forces between —C=O and —NH groups which are believed to result in helical configurations have played a dominant and almost overwhelming part in nearly all the proposed tertiary structures of even the simplest polypeptides. Although the hydrogen bonds between NH— and —C=O groups are important forces, there is at least some reason to question their stability in the aqueous system where biological activity is nearly always encountered. Edsall and Wyman[2] suggest the possibility that "in aqueous solutions a helical polypeptide chain is rather likely to unfold into a more open and random type of coil". In a similar manner Schellman[3], from a study of the urea-water system, has concluded that hydrogen bonds, taken by themselves, give a marginal stability to ordered structures, which may be enhanced or disrupted by interactions of side-chains.

These questions about the relative stability of —NH——O=C— hydrogen bonds in the competitive aqueous system have opened the way for the serious consideration of other forces which may be operative under biological conditions to influence the tertiary structure of proteins. The interesting work by Kauzmann[4] concerning the probable effect of the hydrophobic attraction of the non-polar side-chains of peptides or proteins is an example of a new emphasis in the problem of tertiary configuration. His recent review[5] of the forces responsible for maintaining the native configurations of proteins includes a more complete exposition of the hydrophobic attractions as well as the dispersion forces (London forces) which are closely related to them. Equally provocative has been the extensive work with poly-L-proline[6a–g]. In this polymer all the usual considerations, such as peptide —NH——O=C— hydrogen bonds, disulphide linkages, and electrostatic attractions, are completely absent so far as can be seen, and yet the poly-L-proline exists in two definite stable configurations which are seemingly dependent for their stability upon the solvent systems employed. One stabilizing force which could apparently be present in this polyamino-acid in aqueous solution would be the hydrophobic attractions of the non-polar side-chains. Thus the nearly planar pyrrolidine rings of the L-proline units in a molecular model can be piled above each other like a series of staggered plates with a spiral of carbonyl oxygen atoms going up around the outside of the pile. (Cowan and McGavin[6a] actually indicate that in the pyrrolidine ring the γ-carbon atom is about 0·5 Å. out of the plane.) Since the distance between these carbonyl oxygens on the spiral is such that a water molecule fits between two adjacent oxygens almost exactly in the model, it is perhaps not out of order to consider that such a spiral could be stabilized by a combination of hydrophobic attractions of the rings and hydrogen bonding of water molecules between carbonyl oxygen atoms to yield the so-called *trans* form of poly-L-proline (poly-L-proline II)[6g]. I have prepared such a model, but prefer not to present it for discussion since with available construction kits it is impossible to represent the γ-carbon displacement (0·5 Å. out of the plane of the ring), an item of considerable importance in a structure containing only proline units. It is interesting that poly-L-proline II (stable in the aqueous system) can be converted back to poly-L-proline I by acetic acid–propanol[6d], a solvent system which might be expected to disrupt hydrophobic attractions.

In my work on molecular models I have been most concerned with some of the natural products of intermediate size and well-established structure containing relatively few amino-acids. These smaller polypeptides have frequently been characterized not only with regard to their amino-acid sequences but also in other fine points of structure such as possible ester linkages, ε-amino lysine linkages, and the presence of D-amino-acids. Many of the polypeptide antibiotics and hormones are in this category. Since most of these molecules have interesting biological activity, and lend themselves fairly conveniently to the construction of molecular models, it was hoped that such a study would give some clue to structural similarities other than amino-acid sequence which might be the basis for a common biologically active unit.

Theoretical Considerations

In attempting to define a common structural feature in these highly reactive polypeptides, I first of all considered the possible conformations of the cyclic ring which is present, for example, in a molecule such as oxytocin. This disulphide stabilized structure has 22 atoms in the ring, and the intact cycle is essential for maximum activity in molecules which possess this system. However, in my opinion, one of the interesting structural features in this cycle is the presence of six amide linkages, and the fact that these groupings can be arranged in a strainless hexagonal configuration with the carbonyl oxygen atoms at the corners of the hexagon. For the purpose of illustrating this concept, a molecular model of a relatively simple (and hypothetical) cystine-stabilized structure,

$$NH_2\text{—Cys—(Ala)}_4\text{—Cys}$$
$$\text{—S——S—}$$

is pictured in Fig. 1*A*.

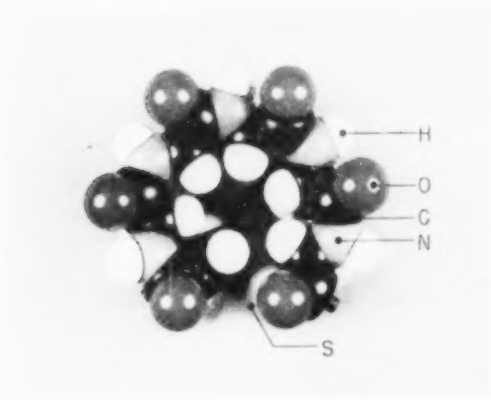


Fig. 1A

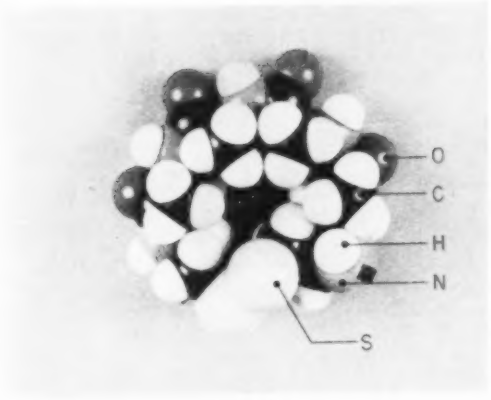


Fig. 1B

This amide side of the hexagon, which I shall refer to as the 'front' or hydrophilic side, shows a very compact arrangement of the α-carbon atoms as a central ring within the hexagon and the α-hydrogen atoms of the L configuration pointing inward to form a nearly circular pattern around an open central area. (In all these models, the absolute configuration for the L-amino-acids as determined by J. Trommel and J. M. Bijvoet[7], was used. All the models illustrated here were made with the Catalin space-filling molecular models (Catalin, Ltd., Waltham Abbey, Essex, England).) The other side of this hexagonal model, which I shall refer to in this and subsequent discussion of other models as the 'back' side or hydrophobic side, is shown in Fig. 1*B*. It will readily be seen that the arrangement of amide-groups suggested for the 'front side' has the consequence of bringing all the non-polar methyl groups on the 'back side' into very close proximity. Thus the possibilities for maximum hydrophobic attractions which are suggested as desirable for an aqueous environment by Kauzmann[4,5] are perhaps more rigidly satisfied by this hexagonal structure than by other possible conformations. In addition to these favourable non-polar interactions on the 'back side' of the model, the carbonyl oxygen atoms on the 'front side' of the plane are almost exactly 4·8 Å. from each other[8], which would be the proper distance to hydrogen bond one water molecule based on an oxygen–oxygen distance of about 2·90 Å. in liquid water (at 1·5° C.) and an H—O—H angle of 109°. Thus the proposed hexagonal configuration of Figs. 1*A* and *B* could be the favoured form in an aqueous environment by virtue of the co-operative combination of (*a*) 'back side' hydrophobic attractions and (*b*) 'front side' hydrogen bonding of water by adjacent carbonyl oxygen atoms.

In joining the disulphide linkage of the cystine unit, we have taken into consideration the most stable conformation of the $—CH_2S—SCH_2$-linkage with a dihedral angle of 90° between the methylene groups as discussed by Calvin[9]. This dihedral angle imposes certain restrictions on the approach distance of the two carbonyl oxygens of the cystine unit and may be a probable factor in the uniform spacing of these two atoms. Other implications of the configuration of the disulphide linkage on chain structure are discussed by Haurowitz[10]. Although Figs. 1*A* and *B* picture a hypothetical polypeptide containing the simplest non-polar side-chain of alanine with a cystine unit in order to present the general principles of our proposal, the models could represent a possible general conformation of the oxytocin ring.

A second consideration in applying this concept of hexagonal structures as probable reactive units can be observed with the cyclic decapeptides such as gramicidin *S* (ref. 11), circulin[12], and tyrocidin *A* (ref. 13) or *B* (ref. 14). In one of the possible conformations, these molecules can be shown to be capable of existing as two fused hexagons somewhat analogous to the six-member and ten-member benzene–naphthalene situation. This approach to the structure of the cyclic decapeptides also offers some interesting clues to the structural necessity of certain D-amino acids, which I hope to point out as the discussion progresses.

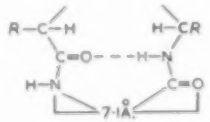
A third consideration in this concept of hexagonal structures as reactive units concerns the open central position in the hexagon, which is readily apparent in Figs. 1*A* and *B*. Since this open central position is in a definite fixed position in the hexagon, it could serve as an opening through which a reactive side-chain group (for example, δ-NH_2 group of ornithine, γ-CO_2H of glutamic acid) could enter the 'front' side of the hexagon at a rigidly determined position. This possibility too will be discussed in greater detail for specific compounds.

Keeping in mind then the three concepts of (*a*) a hexagonal configuration with an amide or hydrophilic 'front' side and a side-chain or hydrophobic 'back' side, (*b*) the possibility that two or more hexagons may appear as fused units and (*c*) a fixed open centre in the hexagon for side-chain reactive groups, I propose to examine the complete models of some of the simpler naturally occurring polypeptides. In particular we are interested in gramicidin *S*, since a proposed conformation for this antibiotic has recently been published[15,16]. I would also like to extend this discussion to the antibiotics etamycin and actinomycin *D* (C_1) for reasons to be presented later.

Gramicidin S

The primary structure of gramicidin *S* is probably one of the best-established sequences of the polypeptide antibiotics[17] and in addition some of the X-ray dimensions and the overall shape of the molecule have been

determined[15]. The proposed conformation of Hodgkin *et al.*[15] as adopted by Schwyzer[16] is based entirely on internal hydrogen bonding to hold the structure together. A model of their conformation is illustrated in Figs. 2*A* and *B*. This suggested structure places the component amino-acids essentially in two parallel chains joined by proline units at the ends, presumably because proline has been frequently associated with directional changes in the polypeptide chain. Only four of the eight possible —NH groups are actually engaged in intramolecular hydrogen bonding. The two proline residues, of course, have no —NH groups, and the other four —NH groups are presumed to be hydrogen bonded intermolecularly. On the 'front side' of this model (Fig. 2*A*) the two side-chains of ornithine are located above the plane of the amide linkages far from any association with other side-chains. Since these two side-chains are not apparently restricted to any position, the reactive amino-groups will not have a definite fixed place in this conformation. When the model is examined on the basis of Kauzmann's[4,5] discussion of hydrophobic attractions and dispersion forces, the Hodgkin–Schwyzer conformation[15,16] seems to offer little opportunity for maximum hydrophobic interaction of the alkyl side-chains. Not only is there considerable open space between non-polar groups on the same side of the principal plane of the molecule, but also some side-chains are actually on opposite sides of the plane. In fact, it would appear from the models that, in general, the *trans* configuration of the amide-group is less favourable for maximum hydrophobic attraction of side-chains on adjacent amino-acid units since the α-carbon atoms carrying the side-chains are always farther apart when the amide linkage is *trans*. In a similar manner, although Kauzmann indicates that aromatic rings will be in the most favoured position when they are close together, their configuration[15,16] finds the two benzene rings of the respective D-phenylalanine units as far apart as they could possibly be, at the opposite ends of the parallel chains. The structure indicates no particular geometrical advantage in having the D-form of phenylalanine instead of the L-isomer at that position. The overall dimensions of the model do not seem to be particularly close to the rectangular dimensions of 11 Å. × 19 Å. suggested by Hodgkin's X-ray data[15]. Indeed, if one lays out diagrammatically the distances involved between the two parallel chains (taking a liberal value of 3 Å. for the —O——H—N hydrogen bond):



then the average width of the proposed rectangle is about 7 Å. rather than the 11 Å. value determined by the X-ray method. One or two of the unbonded carbonyl oxygens may be about 10 Å. apart, but that would represent the maximum width at one or two places only. The overall length of the model as shown would correspond to a value of about 24 Å., although this could be varied somewhat by pointing the benzene rings downward in an isolated position.

As an alternative suggestion for the conformation of gramicidin *S*, I would like to discuss the structure which is indicated in Figs. 3*A* and *B*. The formulation of this model has been based on my previous

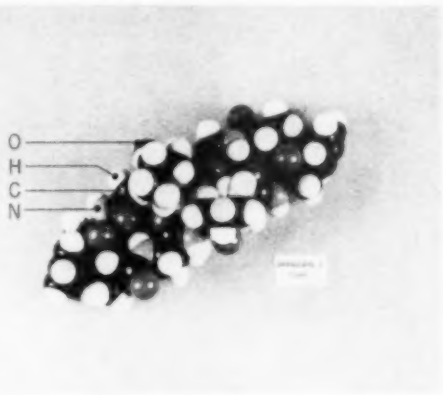


Fig. 2*A*

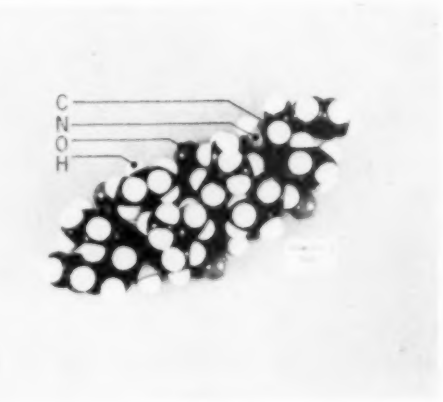


Fig. 2*B*

observation that the cyclic decapeptide ring can be readily arranged in the shape of two fused hexagons, a virtually strainless structure employing the usual bond-angles. In this fused system, the carbonyl oxygen atoms again form the corners of the hexagons, and the α-carbon atoms form two closely knit rings within the individual units. Now in a model of such a cyclic decapeptide, if all the amino-acids were L-amino-acids, the α-carbon atoms would project only a ring of α-hydrogen atoms toward the centre of the molecule, and at the fusion point of the two hexagons there would be a considerable open space. However, in the case of the gramicidin *S* decapeptide there are two D-phenylalanine molecules in the cycle just five units apart. If these two components are placed at the juncture position of the two rings, the opposite D-configuration at that position projects its non-polar side-chain (instead of the single α-hydrogen atom) into the central open area and the open space is neatly filled. Indeed, the —CH_2— groups of the two D-phenylalanine side-chains in this suggested conformation so direct their respective benzene rings into the central space that the two rings are exactly side by side. Not only does this projection of the side-chains completely fill the open fusion junction but it also fulfils elegantly Kauzmann's suggestion[5]

that "the aromatic rings of phenylalanine will prefer to be close to one another" in the most desirable hydrophobic arrangement. The smaller central open area in the individual hexagonal units is still available for the entrance of the δ-amino ornithine groups from the 'back side' of the structure. It will be noted that, if one prefers to presume that proline units are associated with a change in the direction of the polypeptide chain (a point which may still be open to question), then the proline units adjacent to the D-phenylalanine units are also located at the proper positions. In this conformation a look at the 'back side' (Fig. 3*B*) of gramicidin *S* is even more revealing, since it clearly shows the attractive possibilities for maximum hydrophobic bonding. With the two benzene rings fitted nicely side by side, these aromatic substituents are also flanked by the nearly planar rings of the two proline units to form four compact layers. On either side of this central compact layer we have the non-polar groups of valine, ornithine, and leucine fitted closely together with maximum hydrophobic attraction. The δ-amino groups of the two ornithine side-chains are thus brought firmly into position in the two small open centres of the individual hexagon units, and appear in a fixed position in the 'front side' of the structure (Fig. 3*A*). The general shape of the 'back side' of gramicidin *S* in our proposed model is roughly rectangular. Because of the layered arrangement of the pro-phe-phe-pro side-chains it is possible to calculate the width of this rectangle from the standard approach distances of aromatic nuclei and the van der Waals's radii of methylene groups[18]. This calculated value is 10·8 Å., very close to the observed value of 11 Å. The length estimated from the Catalin model is about 16–17 Å., which is not in complete agreement with the observed value of 19 Å. However, these dimensions do not take into account the possible contribution to the overall size by three moles of water and two moles of ethanol known to be present in at least one of the crystals examined[18]. I would like to emphasize again that I am proposing this conformation as the one which might be stabilized by forces operating in an aqueous system, which may or may not be different in their magnitude or direction from forces operating in the crystal state or in a non-aqueous environment.

The points which seem to favour our conformation of gramicidin *S* (Figs. 3*A* and *B*) may be summarized as follows:

(*a*) The overall size and shape of this conformation as indicated from the model and actual contact distances seem to fit the X-ray data perhaps somewhat more satisfactorily than the previously proposed conformation of Hodgkin and Schwyzer[15,18].

(*b*) The structure gives maximum aggregation of hydrophobic groups, together with a most favourable placing of the aromatic rings of the D-phenylalanine units.

(*c*) The fused hexagon arrangement indicates a probable specific structural advantage or requirement for the presence of the two D-phenylalanine units in the molecule, a point that is not so apparent in the alternate arrangements thus far proposed.

(*d*) The smaller open central areas in the two hexagonal units provide spaces for the rigid arrangement of the two δ-amino-ornithine groups at fixed distances from each other on the 'front side' of the molecule. This 'front side' may be regarded as the hydrophilic side of the structure, and may be the reactive surface of the molecule.

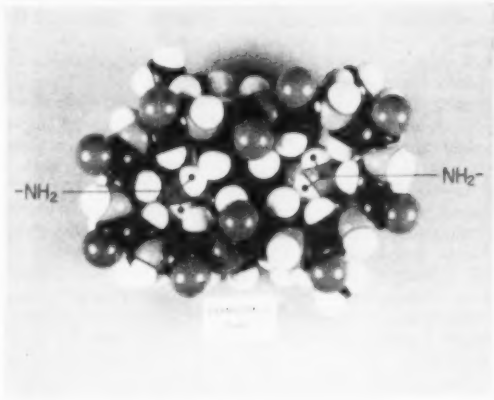


Fig. 3*A*

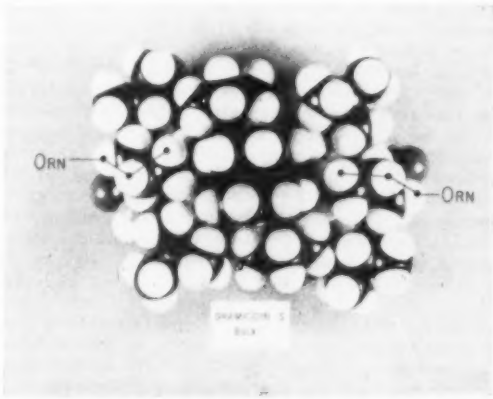


Fig. 3*B*

One other feature of the 'front side' (Fig. 3*A*) of gramicidin *S* may be worth mentioning. Schmidt[15] emphasizes in a discussion of the X-ray data that "there appear to be many groups about 4·8 Å. apart". Similar reflexions of approximately 5 Å. were observed by Cowan and Hodgkin[18] in their work with insulin and gramicidin *B*. It may be suggested that eight of the ten oxygen atoms at adjacent corners of the fused hexagons are approximately 4·8 Å. apart as estimated from the models and from diagrams based on measured angles and interatomic distances. The two centrally located amino groups are also at a similar distance from the carbonyl oxygen groups.

Etamycin and Other Polypeptides

The complete lack of —NH groups already noted for poly-L-proline is a feature which is shared to a somewhat lesser extent by at least two classes of naturally occurring polypeptide antibiotics. Thus, for example, in etamycin[20] and the whole spectrum of actinomycin antibiotics[21*a–d*] a majority of the amino-acids in the molecule have secondary amino groups (such as sarcosine, N-methylvaline and others) which prevent these units from participating in —NH

bonding with other carbonyl groups. Therefore the tertiary structure of these compounds is more probably determined by other forces such as hydrophobic bonding, at least when compared with compounds having a full quota of —NH groupings. Indeed, as if to augment the low number of —NH bonds, the percentage of amino-acids with non-polar (that is, hydrophobic) side-chains is also very high in etamycin as well as the actinomycins, so that both effects would operate in an aqueous system toward configurations with a high degree of possible hydrophobic bonding. In these two types of structures, salt linkages and disulphide bonds are also absent. Both compounds also contain D-amino-acids which I think may be structurally expedient to promote closer hydrophobic fitting as in the case of gramicidin *S*.

Using these concepts of maximum hydrophobic bonding in aqueous systems, I have prepared a model of etamycin and photographed it from the 'front' (amide side) in Fig. 4*A* and the 'back' (hydrophobic side) in Fig. 4*B*. Fig. 4*A* shows the overall compact configuration which this proposed arrangement permits. Into the centre of the hexagonal unit it brings the phenolic hydroxyl group of the 3-hydroxypicolinic acid, held in that position by the benzene ring of phenylsarcosine. It will be noted that, with the exception of the ester-linked L-threonine, each of the other L-amino-acids of etamycin contributes its single α-hydrogen atom to the central ring surrounding the hydroxyl group of the hydroxypicolinic acid. However, the D-allohydroxyproline, having the opposite configuration, points its β-methylene group into the central area, which also brings the γ-carbon containing the hydroxyl group alongside the pyridine ring of the picolinic acid. This hydroxyl group of the D-allohydroxyproline assumes a very interesting and significant position in the proposed conformation. First of all, it lies immediately adjacent to the nitrogen atom of the pyridine ring; and consequently the —N——H—O— bonding observed by Pressman[22] for water molecules with the ring nitrogen of *iso*nicotinate, nicotinate, and picolinate, will be a highly probable attraction. When the hydrogen atom of this hydroxyl group is pointed toward the ring nitrogen, the oxygen atom is turned in the proper direction so that it can hydrogen-bond very neatly on its 'back side' with the —NH group of the picolinate–threonine amide bond as well as the —NH group of the threonine -D-leucine bond. Some of these possible interactions are seen more clearly on the other side of the model (Fig. 4*B*). The —NH grouping of the D-leucine amide link to threonine is best accommodated in the ring structure in the *trans* configuration. With two D-amino-acids adjacent to each other in the etamycin chain sequence, steric interference permits only one of the side-chains to be arranged in the central area of the hexagon. The three probable interactions which were mentioned for the hydroxyl group of D-allohydroxyproline strongly favour its inclusion in the central area instead of the D-leucine. As a consequence the rather bulky non-polar side-chain of D-leucine is pushed to the outside, a situation which is best accomplished by the indicated *trans* amide linkage at the threonine-D-leucine junction, and then simultaneously favoured by the resulting hydrogen bond with the hydroxyl group of its D-allohydroxyproline neighbour. So far as the 'front side' of the conformation (Fig. 4*A*) is concerned, the presence of two adjacent D-amino-acid residues produces a 'change of direction' in the amino-acid chain so that the carbonyl oxygen of D-leucine is crowded outside the hexagon formed by the other six carbonyl oxygen atoms.

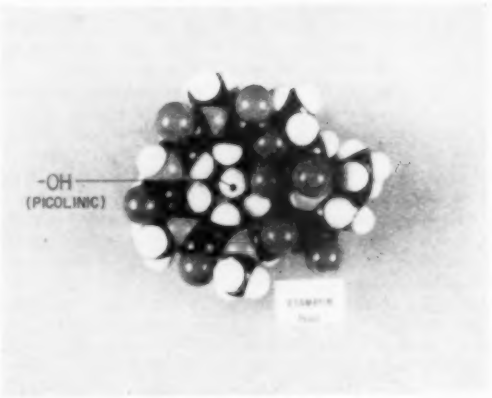


Fig. 4*A*

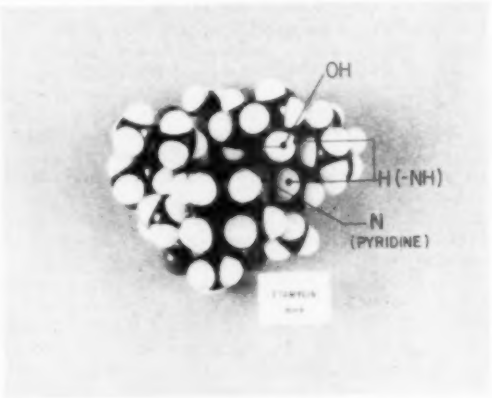


Fig. 4*B*

Other features of the hydrophobic 'back side' of etamycin (Fig. 4*B*) are the very close fitting of the aromatic rings of benzene (phenylsarcosine) and pyridine (picolinic acid) placed side by side, and the other non-polar groups compactly arranged around the two rings. The D-allohydroxyproline ring is particularly well fitted against the pyridine ring, and the entire conformation agrees well with Kauzmann's suggestions[5] about hydrophobic bonding and dispersion forces. In this proposed conformation of etamycin, hydrophobic attractions and hydrogen bonding possibilities can both be present to a maximum extent, although the suggested bonding would not involve a carbonyl oxygen, but the hydroxyl group of D-allohydroxyproline. This model of etamycin also shows quite nicely that the hydroxyl group in the D-allo isomer is in a better position for hydrogen bonding than the hydroxyl group of the more common hydroxyproline.

The third molecular model I shall discuss in detail is actinomycin *D* (C_1). From the work of Brockman *et al.*[21a–c] and Bullock and Johnson[21d], the actinomycins have been shown to contain a substituted phenoxazine chromophore attached to two

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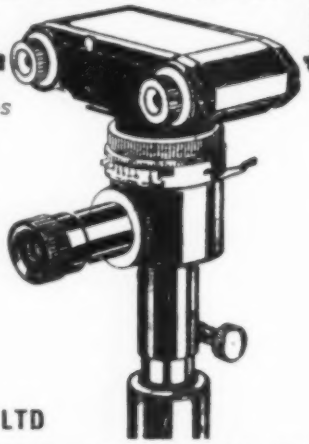

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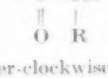
individual cyclic pentapeptide lactone rings through the amino-group of threonine, while the hydroxy group of threonine forms the lactone juncture. Models of the structure of actinomycin *D* as it appears in the literature can be made; but the two pentapeptide rings are quite strained, and there is, of course, a certain amount of 'free rotation' about the amide bond of the threonine–chromophore junctions. Consequently the entire conformation has little structural rigidity and a low degree of hydrophobic interaction, especially between the chromophore and the non-polar side-chains. However, if one examines the chemical evidence for the two individual lactone rings, the methanolic sodium hydroxide hydrolysis[23], consuming two equivalents of alkali, is equally consistent, in my opinion, with a decapeptide dilactone ring in a continuous cycle and attached at two points to the chromophore. Similarly, the alkaline peroxide degradation of the chromophore[21d] to yield two pentapeptide fragments would proceed equally well from a decapeptide dilactone. Even the recent synthesis of actinomycin C_3 by Brockman and Lackner[24] is not unequivocal proof for the two individual pentapeptide lactone rings. Schwyzer and Sieber[25] have demonstrated previously that the pentapeptide, val-orn-leu-phe-pro (L,L,L,D,L), preferentially forms the cyclic decapeptide, gramicidin *S*, instead of the expected cyclic pentapeptide. Consequently the formation of the decapeptide dilactone should be considered in the actinomycin C_3 synthesis. It is interesting that Brockman[26] also considered the possibility of the decapeptide dilactone in his earlier work, but discarded it later because of his views that the chromophore is formed biologically from the oxidative fusion of the pentapeptide lactone of 3-oxo-4-methyl-anthranilic acid[21c].

Because of the distinct possibility that the actinomycin molecule may be a decapeptide dilactone, I would like to present a model of this alternate structure for consideration as a very likely primary structure and conformation of actinomycin *D* (C_1). The suggested amino-acid sequence of the decapeptide dilactone is given in Fig. 5.

The positions of the abbreviated amino-acid symbols correspond approximately to the carbonyl oxygen positions of the amino-acids in the molecular model (Fig. 6*A*), with the



direction counter-clockwise. In the case of the threonine, the —NH points toward (and above) the chromophore while the —OH forms the lactone with N-methylvaline.

The molecular models of actinomycin *D* (C_1) are illustrated in Fig. 6*A* ('front side') and 6*B* ('back side'). This proposed structure for actinomycin places it in the same class of compounds as gramicidin *S*, circulin and the tyrocidins. In the form of the decapeptide dilactone, the component amino-acids surround and support the chromophore whereas in the alternate case of two pentapeptide lactones these rings circled above the chromophore. The decapeptide dilactone therefore gives maximum hydrophobic interaction. This is apparent also from the 'front side' (Fig. 6*A*). The two molecules of D-valine bring the —CH and one CH_3 group of the side-chain into the central open space next to the chromophore, thus effectively shielding this resonating system as well as supporting

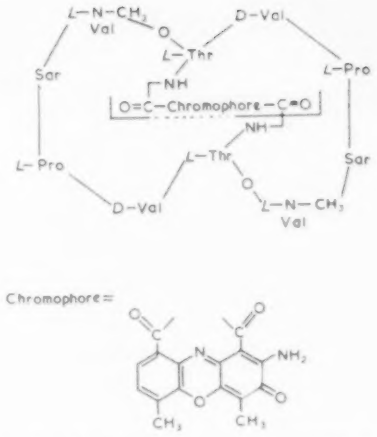


Fig. 5. Probable amino-acid sequence of actinomycin *D* (C_1)

it in the central portion of the molecule. (Substitution of L-valine in the model gives a much less satisfactory fit.) Thus, our proposed configuration of the molecule indicates again a definite, structural, space-filling preference for the D-amino-acid. This utility was also suggested for the D-phenylalanine units in gramicidin *S* and for the D-allohydroxyproline and D-leucine in the case of etamycin. It will be noted that here, too, as in the etamycin and gramicidin *S* models, the D-amino-acid is associated with a change in direction of the amino-acid chain; for in order to accommodate the bulky D-valine side-chain in the centre of the structure, the threonine carbonyl group points toward the centre and consequently projects its side-chain outward toward the ester linkage with N-methyl-L-valine. Although actinomycin *D* has only four possible —NH groups for hydrogen bonding, it will be noted that two of these —NH——O=C— hydrogen bonds are nicely formed between the —NH group of one threonine unit and the carbonyl oxygen of the other threonine unit at the junction of the two hexagons.

With the rather rigid chromophore grouping in actinomycin *D*, it should be possible to measure quite accurately the distance between the carbonyl oxygen atoms at the centres of the decapeptide dilactone. Although interatomic distances for phenoxazine are not recorded, a fair estimate may perhaps be obtained from the values for phenazine and benzoic acid[27]. The value so obtained would be about 7·2–7·4 Å., depending on the angles selected between the carbonyl group and the aromatic ring. The value estimated from the Catalin models is about 7·3 Å. (measured tangentially to the two carbonyl oxygen atoms).

The hydrophobic 'back side' of the actinomycin *D* (C_1) (Fig. 6*B*) shows a central location of the chromophore. The symmetry of the picture is quite apparent, being roughly rectangular or elliptical in shape with matching corners and sides. Here, too, the resonating chromophore system is seen to be well protected by non-polar, hydrophobic groups, almost like an electrical insulating layer. I should perhaps emphasize again that this structure would, in our opinion, be a probably preferred configuration for the aqueous (or biological) system, and might not be present with solvents which would rupture hydrophobic bonds. This 'back side' of actinomycin *D*

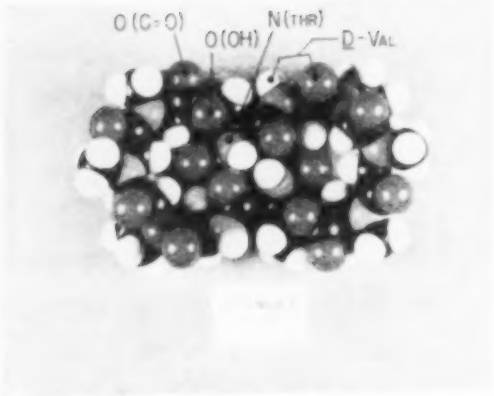


Fig. 6A

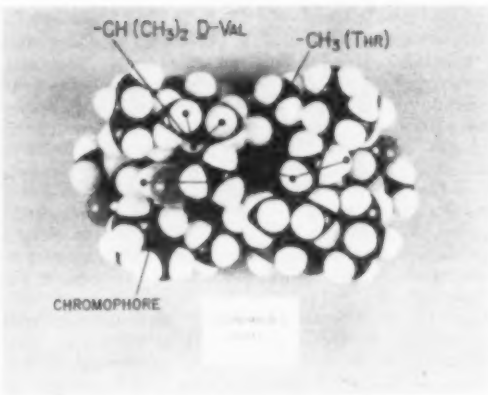


Fig. 6B

shows a slight open space between the threonine methyl group (which was purposely moved slightly away from the chromophore to pinpoint its position) and the adjacent methyl group of D-valine. When this particular valine methyl group is replaced by an ethyl group, the space along the sides of the chromophore is even more uniformly filled, and the resulting amino-acid turns out to have the structure of D-allo-*iso*leucine[32]. It was of interest to us that the configuration of D-allo*iso*leucine was predicted from consideration of the open space in the model before we had checked the actual assignment of the structure, but this result could, of course, have been fortuitous. The model containing two moles of D-allo*iso*leucine would be actinomycin C_3.

One other feature of the 'back side' of actinomycin *D* (Fig. 6*B*) is perhaps worth indicating in this conformation. The long, slender shape of the chromophore group requires as much space as possible at the ends to accommodate the benzene rings and especially the amino substituent of the phenoxazine nucleus. This space is conveniently provided in this particular conformation by having sarcosine units with their minimum-sized α-CH_2 groups exactly in line with the ends of the chromophore at that point. The rigid arrangement of the ends of the chromophore relative to the sarcosine units may also be an important point in the recent acid degradation studies with actinomycin C_3. In these experiments[28a] concentrated hydrochloric acid at 37° selectively splits the molecule in only one place at a sarcosine–methyl valine linkage. From Fig. 6*B* it is apparent that the sarcosine–methyl valine juncture at the left end of the chromophore is near the chromophore amino group, but the similar amide bond at the right end of the chromophore does not have a similar neighbouring group. Such adjacent amino-groups are known to contribute an increased acid stability to the glycosidic linkage in neobiosamines[29] and we have observed a similar stabilizing effect in the very slow acid degradation of the glycosamine polymer, chitosan[31]. Consequently this particular decapeptide dilactone conformation also offers a plausible explanation for the selective acid hydrolysis of one of the sarcosine–methyl valine bonds and the relative stability of the other.

In the course of our work with these molecular models we have prepared similar proposed conformations of other structures such as the complete oxytocin molecule and the cyclic decapeptide, circulin. The circulin model may be especially interesting because it shows how a single D-amino-acid (D-leucine) can effectively fill the open centre of the two fused hexagons which may be the preferred conformation for the cyclic decapeptide antibiotics. The recent disclosure of the formulæ of circulin *A* and *B* by Koffler and his associates[32a,b] has prompted me to include a single model (Fig. 7) showing mainly the decapeptide ring, with only the α-hydrogen atoms (but not the side-chains) of the nine L-amino-acids, and the $-CH_2CH(CH_3)_2$ side-chain of the D-leucine. The D-isomer side-chain may also function as a structural spacing unit to separate the two central carbonyl oxygen atoms. This may also be true for the two D-phenylalanine side-chains in gramicidin *S*.

Concluding Remarks

It will, of course, be appreciated that the proposed 'fused hexagon' conformation which has been discussed for cyclic decapeptides of the antibiotic type may also be capable of extension to the active centre of an enzyme, such as trypsin, where a disulphide-stabilized decapeptide is presumably part of the reactive unit[33]. If the two reactive centres in such a trypsin cyclic decapeptide are at approximately the same distance as the two centres of the actinomycin chromophore (about 7·3 Å.), then it could perhaps be significant that the distance between

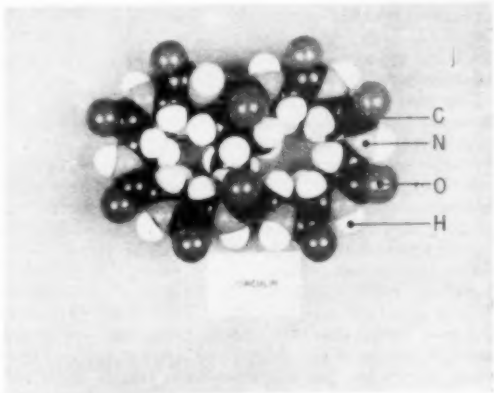


Fig. 7

the ε-amino group of lysine and the carbon atom of its carbonyl amide group (where anion attack in hydrolysis might be expected) is definitely approximately 7·2–7·5 Å. as judged by the Catalin model of the extended chain. This dimension was also estimated at 7·4–7·5 Å. diagrammatically from the interatomic distances and angles given for DL-α-norleucine (ref. 27, *M* 207), arbitrarily using the α-NH_2 angle with the α-C atom as a rough approximation for the angle of the ε-amino group with the ε-carbon atom in the extended chain configuration. Such a space separation of the reactive groups would allow attachment of the ε-amino group of lysine at one point and hydrolysis of the amide linkage at the other point. With the incomplete information thus far available concerning the active centre of trypsin this is admittedly a very speculative consideration, but the indicated dimensions are at least consistent.

Our considerable experience with these models in the construction of decapeptides and other slightly smaller polypeptides substantiates clearly the points raised by Platt[24] in his discussion of the need for better models. We noted difficulties especially in the gramicidin *S* model, where the bulky groups had to be held in place by small bolts and rubber bands to simulate hydrophobic attraction. As a result small spaces sometimes appear between the joined atoms in the photographs which are the result of mechanical weakness of the model bonds and slight differences in bond-angles rather than steric hindrance in the actual structure. No doubt these difficulties can be surmounted, and the problem is strictly an economic problem so far as the model suppliers are concerned. In spite of these difficulties, the models have been a great aid in visualizing the conformations which I have presented for gramicidin *S*, etamycin and actinomycin *D*. I think that the conformations show quite clearly the compact hydrophobic attractions which can be present in molecules with fairly complex side-chains, and therefore suggest that these conformations may probably be favoured by the aqueous, biological systems. Whether *cis* amide bonds (allowing the closest hydrophobic attraction of non-polar groups) or *trans* amide bonds (which seem to be at present favoured but offer less compact hydrophobic attractions) are actually present in the aqueous system is probably a major consideration. The comments by Pauling[25] concerning the probable instability of the *cis* amide linkage which is associated with the presumed orbital overlap of α-hydrogen atoms were based on a van der Waals's radius for hydrogen of 1·2–1·4 Å. This argument should perhaps be re-examined on the basis of the somewhat smaller 'interference radius' of hydrogen which some workers regard as a more accurate measure of possible steric hindrance[26a–d]. The models also clearly show that, in a cyclic hexapeptide with *cis* amide linkages, the carbon–hydrogen bond at the tetrahedral α-carbon atom projects upward at about a 20° angle with respect to the main plane of the hexapeptide ring. Consequently a C—H distance of 1·07 Å. is equivalent to an 'in-plane' projection of about 1·00 Å., so that any crowding of neighbouring α-hydrogen atoms is further relieved. In the light of these considerations the *cis* configuration of the amide bond may be consistent with a desirable close-fitting structure instead of a sterically unstable conformation. Its presence is certainly consistent with maximum hydrophobic interactions which may be highly effective in the aqueous system.

I wish to thank many of my colleagues at the Upjohn Co. for their helpfulness in the preparation of this manuscript and their encouragement of the work. I am particularly grateful to Prof. C. H. Li for his suggestions and interest. I want to thank Mr. Eugene Beals of our photography group for his patient help in preparing the pictures of the molecular models. The co-operation of Mr. J. E. S. Whitney of Catalin, Ltd., in preparing the special model atoms of peptide nitrogen for use in constructing these conformations is appreciated.

Note added in proof. Since this manuscript was submitted for publication, papers, by Klotz and Franzen[27] on the stability of interpeptide hydrogen bonds in aqueous systems, and by Tanford *et al.*[28] on hydrophobic bonds and the structural stability of native proteins, have appeared which are pertinent to the validity of the conformations proposed.

With regard to Figs. 3*A* and 3*B*, the possibility that the L-leu-D-phe amide junction may involve a *trans* amide bond with the —NH— pointing toward the hexagonal centre is suggested. Similar considerations could apply to Fig. 7 and minor changes in the arrangement of the D-leu unit in Figs. 4*A* and 4*B* might improve the hydrophobic picture.

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THE TECHNOLOGICAL UNIVERSITY

By Dr. S. C. CURRAN, F.R.S.
Royal College of Science and Technology, Glasgow, C.1

School Education

OUR schools, particularly the Scottish schools, are in a critically dangerous state so far as the flow of properly trained students to the universities is concerned. This is affecting the number of entrants to science and engineering, which are the principal fields of study in a university biased towards technology. Early action of a radical kind is required if pupils in our senior secondary schools are to be given a fair opportunity to enter the most expanding part of the national education scene. Most schools seem to be desperately short of staff adequately trained in mathematics and physics, and to a lesser extent chemistry. The two fundamental sciences of physics and chemistry must be given the status of full subjects and staffed accordingly as otherwise physicists are penalized.

The length of training, four years in an honours course, followed by a year of study at a training college, is much less attractive than the shorter passage to teaching in England. The deadlock must be broken, as otherwise Scottish pupils will suffer severely by missing the opportunities available to them in science and technology. Salary differentials of an attractive nature might be one answer, and this might be effected by the special staffing of advanced-level schools set up at chosen centres. These schools could take pupils from approximately the Ordinary Level to the Advanced Level and the Scottish and English patterns thus brought nearer to equivalence in spite of the special difficulties encountered in Scotland. These stem to a large extent from the fact that the population is more widely dispersed so that the sixth form is a sham in most instances. It is impracticable and very expensive to set up good sixth forms in the majority of Scottish schools.

The higher-level education in technology depends on school performance in mathematics, physics and chemistry, and the drive towards specialization in these subjects in the English schools has been examined recently by scientists who advised some reduction in extreme specialization in all fields. In Scotland there is no specialization in this sense, and whereas the advice offered to English schools was to cut the study in science or classics or any other single specialized field to half the total, the Scottish schools simply do not, indeed can not, offer that much. If they did the staffing position would become still more desperate.

Some outlet for the natural appetite of pupils of 16–18 years of age must be offered. They are then at one of the peaks of their mental activity, they are intensely interested in fashioning their future career and the lack of adequate facilities for the young people in the upper reaches of our schools is bound to depress and thwart them. Many are being lost and more will be lost unless we find a plan and are prepared to carry it out immediately. There is no reason to decry the wish of the young people to choose their subjects with a vocation in mind. Interest is the sure key to learning and in turn to understanding. If the right subjects are taught well it is all to the good if the vocational aspect is obviously attractive. This is one of the great points of strength of mathematics and science. They are necessary for entry to so many fields in which trained men and women are required.

A Modern University

There seems to be an obvious need for a form of university that is not, so far, part in its own right of our British university system. This might be termed the technological university though this is something of a misnomer. If one takes a thoroughly acceptable form of definition of a university training it is clear that an education in science and engineering of the right kind satisfies the conditions extremely well. A proper understanding of these subjects is both cultural and vocational at one and the same time. The technologist requires too a 'rounding-off' of his education and this can be very appropriately provided by study of the social sciences or humane studies in which a keen interest is for him a natural extension of his more professional studies. This is recognized in universities of this type, for example, the Massachusetts Institute of Technology, where those responsible for the courses in the social studies are of the highest calibre. In the hands of such real experts in their fields the courses are of great value. In such a university, too, the reverse is readily possible although not so frequently practised. A study of the social sciences with a good grounding in science or engineering offers an excellent kind of degree course.

Such an institution has great strength in its freedom from some of the difficulties facing the

• Substance of a lecture delivered before the Royal Society of Edinburgh on March 6.

really comprehensive multi-faculty universities. The administration of those is a task of great difficulty, but there could be some uniformity of purpose in the more modern form of university. This could enable it to organize more easily inter-departmental programmes at both undergraduate and postgraduate level.

Research

At present there is great danger that universities may lose their traditional role of leadership in fundamental research and this might well be taken over by large research groups under governmental control. A university operating in science, engineering and the social sciences has a form lending itself to the proper handling of real research programmes, and so research leadership of a more valuable type could be retained.

Such a university gives its graduates a real appreciation of the wholeness of our Western civilization. The departments would have a real sense of their common aim. The cultural target would be clearly established. The subjects would not be treated as segments of our intellectual heritage operating in comparative isolation, a situation not uncommon in our universities.

The position in Britain is not at all satisfactory. In the United States and on the Continent of Europe the situation has been faced squarely during the past century. Large institutions devoting themselves to science and technology and associated fields such as the humane sciences have long granted their own degrees and stood alongside other types of university. Thus we have, for example, the Massachusetts Institute of Technology, and institutes of comparable standing at Zurich and Aachen. The industrial strength of the nations depends more and more on such institutions and they are an absolutely vital part of the higher educational system. We ignored the vital role of science and scientific research so that France and later Germany far excelled us. About a century ago we finally decided to turn professional and leave amateurism in pure science, and our universities admitted the sciences. But applied science and engineering have never been adequately recognized and we must act now, see to the full recognition and acceptable status. This will mean that the training of scientists and technologists can be organized on an adequate scale to satisfy the vital needs of Britain for graduates and for thoroughly good research.

The number of scientists and engineers required is such that the balance of the orthodox form of university structure would be upset. Some five or so applied scientists are required for each pure scientist; but the faculty of science itself is usually large as faculties stand. Clearly it would be wise to recognize that the real demand cannot be met by existing universities, and that in meeting it well it is wise to set up universities of a pattern suitable to meet the need in the most appropriate way. The new institution will more readily provide the men with the type of education that is truly of the best university type even if the institution is not of the orthodox pattern of universities with comprehensive subject coverage. It has as its primary objective the provision of a true university education in a limited number of fields without the weaknesses inherent in one and the same place attempting to provide it in all fields. Conditions are such that to try to do so in all fields, especially by including technology as one of the many, is to court disaster.

We live in a technological age, and the number of technologists required should only be limited in the future by the number of those with the adequate capacity for the subjects and the taste for them. Given an education of an integrated type the men graduating from such a university will scarcely be aware of the so-called division between the two cultures. They will have learned to absorb both and should continue to do so throughout their lives. These are the views which hold sway at the Royal College of Science and Technology, Glasgow, and we have applied them already in our courses and we will apply them increasingly in the future.

SIGNIFICANCE OF CARBONACEOUS METEORITES IN THEORIES ON THE ORIGIN OF LIFE

By Prof. J. D. BERNAL, F.R.S.

Birkbeck College, University of London

At the meeting of the New York Academy of Sciences on March 15, 1961, B. Nagy, W. G. Meinschein and D. J. Hennessy reported their findings of mass spectrographic analysis of carbonaceous material from the Orgeuil meteorite of 1864 (*Nature*, 189, 967; 1961). They concluded from this that the distribution of hydrocarbons in the high-temperature distillate was so similar to that found for animal products such as butter or organic materials in sediments as to justify the conclusion, admittedly preliminary, "that biogenic processes occur and that living forms exist in regions of the universe beyond the earth".

It was this conclusion that, quite naturally, gave rise to much publicity. There is a danger that this will, in serious scientific circles, cause the observations of the three authors to be overlooked, which would be a great mistake, for whatever the interpretation put on them they are of cardinal importance to science.

It would be premature to discuss here the detail of these observations in advance of the publication of the full report; but enough has appeared to understand their general tenor, and it is important that they should be widely known, for a reason which will be discussed more fully later, otherwise careless study of other meteoritic material may well destroy irreplaceable evidence.

The study of carbonaceous material in meteorites is not new. The first detailed analysis to be published

was that of G. Mueller in 1952 on the Cold Bokkevelt meteorite, which seems similar to that in the Orgeuil meteorite. Using conventional methods of analysis, he showed that a large part of the black material was soluble in organic solvents and the recovered material showed a somewhat unusual composition: hydrogen, 8 per cent; carbon, 24 per cent; nitrogen, 4 per cent; oxygen, 49 per cent; sulphur, > 9 per cent; chlorine, 6 per cent. The high oxygen content suggests a complex organic acid. If it was to be compared with any material produced by living systems, it would be with the humic acid of peat. Mueller drew no cosmic conclusions from his work, and it attracted little attention at the time.

The recent work is consistent with this picture but completes it in a significant way. Using mass spectrographic analysis of a distillate of the meteorite, they found the largest portion of the 250°–400° C. fraction to consist of paraffinoid hydrocarbons of carbon numbers ranging from 15 to 24 with peaks at 18 and 23 carbon atoms, as well as some cycloparaffins. This distribution is similar but by no means identical with that found by the authors for butter and recent sediments using the same apparatus.

There can be no serious doubt that the Orgeuil material is from a genuine meteorite, and that it has been examined in an uncontaminated state. The mass spectroscopic evidence should also be accepted in view of the experience of Dr. Meinschein in precisely this type of work in the petroleum field.

The major question that arises, therefore, is as to the interpretation of the results. Though the mass spectroscopic evidence indicates a composition of meteoritic hydrocarbons within the range of those given by material of organic origin on the Earth, from this it only follows that the meteorite material *may* be of organismal origin and not that it *must* be so. The actual hydrocarbon fragments are admittedly artefacts produced from some more complex source by the rather drastic distillation and electron bombardment processes. However, as there is little possibility that they are synthesized by these processes, the original material must contain paraffinoid elements similar to those produced in living matter.

Here the onus of proof is on synthetic chemists who must show that these have been, or could be, produced by purely inorganic condensations from smaller carbon-containing molecules. A possible mechanism for this is suggested below but requires experimental test.

The vital question could be settled once and for all if specific molecules such as porphyrins or nucleotides were found. A counter indication at the moment is the extremely low phosphorus content of the meteorite.

Pending a settlement of this question by analytical or synthetic methods, it may be worth while, in order to direct further experimentation, to speculate on alternate hypotheses for the formation of carbonaceous material in meteorites. These alternatives are either that they are produced by some form of life or that they are produced by wholly inorganic processes.

If the material is derived from life, this must have occurred on the planetary parent body from which by general consensus we believe meteorites to have been derived by fragmentation.

The time of flight of the Orgeuil stone has not, so far as I know, yet been measured; but it is unlikely to be more than 200 million years on analogy with other meteorites. This would mean that in recorded geological time there must have existed a planet on which life processes had evolved sufficiently to produce long-chain hydrocarbons.

Meteoritic parent bodies, however, as most recently demonstrated by E. Anders[1], appear to be located in the asteroid belt between Mars and Jupiter, a region where mean surface temperatures are approximately − 50° C. This would not appear to permit liquid water and hence any chemical evolution. Further, even if hot enough they would not be large enough to hold water vapour gravitationally and, therefore, would be dry bodies like the Moon.

The only likely place for the genesis of such hydrocarbons would be a planet the size of the Earth at about the same distance from the Sun. Why not the Earth itself, if we are not allowed any longer to postulate Pythagoras' anti-Earth? The Orgeuil meteorite would then be a piece of primitive Earth shot off some hundreds of millions of years ago and again united to its parent body. Even more fanciful would be the idea that the stone derives from an earth-like planet of another solar system.

If the first alternative appears difficult to accept, the second only avoids some of these difficulties to encounter others. In this hypothesis, following Mason[2], the nuclei of condensation would be the little platelets of hydrous iron magnesium silicate formed directly from the solar gases. Originally, in the cool and opaque dust cloud surrounding the Sun, these would accumulate ice, methane, ammonia and hydrogen sulphide in solid form from the more volatile solar gas components. As the cloud cleared by particle agglomeration, these would evaporate leaving, however, a thin skin of polymerized carbonaceous material produced by the action of cosmic ray and solar particles. In favour of this we have the evidence from Duchesne[3] that carbonaceous meteorites contain free radicals. There is also the observation of G. Mueller[4] that the very similar Cold Bokkevelt meteorite resisted boiling hydrofluoric acid for 140 hr., indicating the complete carbonaceous covering of each small silicate particle.

The crucial question is now whether it is possible to imitate the carbonaceous material of the meteorites by the action of radiation-work here for atomic piles and high-energy machines. If this question can be answered in the affirmative, the carbonaceous meteorites become the *primary accumulation* of elements and free energy for the first synthesis of life *on this Earth*[5] rather than the end products of chemical evolution on some other celestial body, which in any event would simply remove the problem of the origin of life by another great move in time and space.

We can speculate about these problems now, but what is clearly needed is more careful experiment and critical evaluation. Thanks to the pioneer work of Nagy, Meinschein and Hennessy, there is every reason for believing that this will take place in many laboratories throughout the world, but if this happens without planning and co-operation the results may be scientifically disastrous.

There are probably not more than 50 kgm. of carbonaceous meteorite material in the world to-day; it could easily be used up in a year if each investigator looking for one thing at a time—be it helium-3, porphyrin or radicals—uses up a piece of meteorite in a way to make it unavailable to other workers. What is needed is a carefully worked out flow-sheet needing

the minimum of material, so arranged that only the best equipped and experienced workers do the operation of analysis in which they are expert. This would require an overall organization that fortunately is to hand in the Commission on Space Research and in its constituent organizations in other countries.

The study of meteorites is one involving nearly all the disciplines in science and, like the International Geophysical Year, could be another excellent trial ground for co-operation in science.

[1] Anders, E., *Astrophys. J.*, **132**, 243 (1960); *Geochim. Cosmochim. Acta*, **19**, 177 (1960).
[2] Mason, B., *J. Geophys. Res.*, **65**, No. 9 (September 1960).
[3] Duchesne, J., and Depireux, J., *Nature*, **182**, 931 (1958).
[4] Mueller, G., *Cosmochim. Geochim. Acta*, **4**, 1 (1953).
[5] *Nature*, **186**, 693 (1960).

NEWS and VIEWS

Bacteriology at St. Bartholomew's : Prof. L. P. Garrod

For more than thirty-five years bacteriology at St. Bartholomew's Hospital has been associated with the name of L. P. Garrod, who is due to retire in this academic year. During all these years, Prof. Garrod has contributed much in the field of medical bacteriology. Anyone who knows the cramped conditions in which he has worked will be astonished at what he has achieved. Prof. Garrod belongs to a generation in which it was still possible to be a first-class pathologist and bacteriologist, as well as a first-class clinician, and any who remember his lectures on 'worms' will agree that he is also no mean helminthologist. He has contributed many important articles or chapters in almost every branch of medical bacteriology—for example, actinomycosis, staphylococcal infections and endocarditis, to mention only a few. But Garrod has also kept abreast or even ahead of new developments. In recent years he has often led the way in the critical appraisal of new antibiotics and sometimes in exposing exaggerated claims that have been made. He has been especially interested in the validity of laboratory tests for the sensitivity of bacteria to antibiotics, and he has always tried to show how these tests should be employed so as to give information which may be of real clinical value. He has also been interested in the mode of action of antibiotics, in the differences in bacteriostatic and bactericidal activity, and in the development of antibiotic-resistant strains. He has exerted a profound influence on the development of bacteriology during what may prove to be the first phase of the era of chemotherapy, which is now showing signs of passing from a medical to a more biochemical and biological phase. It is to be hoped that Prof. Garrod will continue after his retirement to work in the field in which he has contributed so much.

Dr. R. A. Shooter

Dr. R. A. Shooter, whose appointment to succeed Prof. L. P. Garrod as professor of bacteriology starts in October 1961, has already made his mark in the field of hospital bacteriology. In much of his work he has been closely associated with Prof. Garrod, but in recent years he has developed a special interest in problems of cross-infection and hospital hygiene and epidemiology. This work has been especially important at a time when surgical wards and theatres and medical wards, too, have been invaded by antibiotic-resistant staphylococci and other organisms, and when the first flush of success in the antibiotic era has given place to a more sober realization of what antibiotics can and cannot be expected to do. Dr. Shooter has paid great attention to problems of staphylococci, of masks, of air and dust and of ventilation in the spread of staphylococcal infection. In the past few months Prof. Garrod and Dr. Shooter have combined with Dr. Blowers and Prof. Williams to produce an excellent book on hospital infection. This has met a very real need and is a fitting conclusion to years of fruitful co-operation in this special branch of bacteriology.

Technical Operations at the International Atomic Energy Agency : Dr. P. L. Balligand

Dr. Pierre Louis Balligand has been appointed to succeed Dr. Hubert de Laboulaye as deputy director general in charge of the Department of Technical Operations of the International Atomic Energy Agency. Dr. Balligand, who is a graduate of the Ecole Polytechnique, is at present head of the Division of Large Research Reactors of the French Commissariat à l'Energie Atomique, which was established to run the operation of the test reactor *EL* 2 and the start-up and development of the high-flux reactor *EL* 3. The service will also include the operation of the test reactor *Pegase* at the new French reactor centre at Cadarache. Dr. Balligand was concerned with the organization of a test reactor research group, the function of which was normalizing in-pile experiments and arranging dosimetry and flux measurements studies. This group has collaborated closely with similar efforts in other European countries : an experimental programme is now being organized in Belgium at Mol, and others are being developed in Italy, Switzerland and Yugoslavia. Reactor safety regulations have been a main concern of Dr. Balligand and the development and application of the first provisional safety measures for experimental reactors at Saclay were his responsibility. However, these regulations are now the responsibility of a Reactor Safety Committee, of which Dr. Balligand was a member. Dr. Balligand will commence his new duties towards the end of March, when Dr. de Laboulaye returns to a senior position with the French Commissariat à l'Energie Atomique.

Industrial Engineering at Toronto : Prof. Arthur Porter

Dr. Arthur Porter, dean of the College of Engineering in the University of Saskatchewan, has been appointed the first professor of industrial engineering in the University of Toronto. Prof. Porter is an authority on the electrical control systems which, among other things, make possible large-scale automation of industry. He worked in Toronto during 1949–55 as head of the research division of Ferranti Electrical, Ltd., the company which installed the University of Toronto's first large electronic computer in 1952. Before becoming dean at Saskat-

chewan in 1958, he was professor of light electrical engineering at the Imperial College of Science and Technology, London (see *Nature*, **181**, 1178; 1958). He is the first member of the University of Toronto staff to be associated solely with the new course in industrial engineering, which superseded the engineering and business course two years ago. Until now, the industrial engineering course has been administered and staffed entirely by professors attached to the Mechanical Engineering, Mathematics and other Departments. The new course covers areas of fundamental engineering and science, but is heavily weighted toward mathematics. Its undergraduates study operations research, electronic data processing, control theory, numerical analysis and probability and statistics. They also are introduced to modern work in organizational structure, financial control and industrial psychology.

Agricultural Economics at Nottingham : D. K. Britton

Mr. D. K. Britton, at present general manager, Market and Economic Research Department, Massey-Ferguson (U.K.), Ltd., has been elected to the new chair of agricultural economics at the University of Nottingham as from June 1. Mr. Britton will be head of the Department of Agricultural Economics and a provincial agricultural economist. Mr. Britton graduated at the London School of Economics and shortly afterwards was appointed statistician in the Ministry of Agriculture and Fisheries. From 1947 until 1952 he was a member of the research staff of the Agricultural Economics Research Institute, University of Oxford, after which he accepted an appointment with the United Nations Food and Agriculture Organization in Geneva. In this work he travelled widely in Eastern and Western Europe. In 1959 he returned to the United Kingdom to take up his present position.

Security Measures and Freedom of the Individual

An immediate repercussion of the recent spy trial in London was the announcement in the House of Commons on March 23 that the First Lord of the Admiralty would set up a committee of inquiry to ascertain what security weaknesses existed at the Admiralty Underwater Weapons Establishment and to determine where responsibility lies for such breaches of security as have occurred. The Prime Minister, in replying, showed himself fully aware of the difficulty of holding the balance between national security and the freedom of the individual from perpetual surveillance in a free society. He quoted the Lord Chief Justice's words that ultimate security must depend always on the honesty of those who are put in a position of trust; and if a person suddenly and for gain becomes dishonest, no security measures are adequate. Existing security procedures which apply to the Government Services were reviewed comprehensively by the Committee of Privy Councillors in 1956, and Parliament accepted these procedures as appropriate and adequate for their purposes. They are kept continually under review by the appropriate authorities, and to adopt a different procedure would involve a much greater range of inquiry about the activities of individuals. Nevertheless, Mr. Macmillan undertook to look at the matter again, and to consider whether the procedures laid down in 1956 should be tightened in any way.

The International Union of Geological Sciences

At the twenty-first International Geological Congress meeting held in Copenhagen on August 23, 1960, it was decided to form an international union of geology. The International Union of Geological Sciences was founded by thirty countries at a Constitutive Assembly held at Unesco House, Paris, on March 9–10, 1961. The statutes prepared by the Organizing Committee in Stockholm in December 1960 were ratified, and the following Executive Committee was elected: *president*, J. M. Harrison (Canada); *vice-presidents*, I. I. Gorski (U.S.S.R.), L. Hawkes (United Kingdom), A. R. Lamego (Brazil), J. Lombard (France), B. C. Roy (India); *secretary*, Th. Sorgenfrei (Denmark); *treasurer*, J. A. Dons (Norway). The object of the Union is to promote international co-operation in geology, and of geology with other sciences. Application has been made for admittance to the International Council of Scientific Unions at its meeting in London in September 1961.

Export Problems and the Technical Chemist

A booklet issued by the Association of British Chemical Manufacturers (86 Strand, London, W.C.2), entitled *European Free Trade Association: Origin Rules, Certification and Segregation of Stocks*, comprises a report of meetings in London and Manchester in June 1960. It includes addresses by W. A. M. Edwards on recent developments concerning the European Free Trade Association and the Common Market, by W. G. Harrold on the need for origin rules and by H. W. Vallender on the operation of the value rule and the associated basic materials; and also N. E. Wallace's paper on the operation of the process rules for organic chemicals, and for other sectors of the chemical industry, and those of L. J. White on the certification and control of the origin of goods and on the segregation of stocks of materials by accounting methods. There is also a note on the provision of information on origin of chemicals purchased for use in the manufacture of products for export to the European Free Trade Area. Intended primarily to assist those concerned in these export problems, these papers, and particularly that of N. E. Wallace, illustrate the technical difficulties with which the chemical manufacturer has now to contend. There is also a short bibliography of official publications, including the key document "Classification of Chemicals in the Brussels Nomenclature 1958".

Funds for Research and Development in Industry in the United States

A survey, *Funds for Research and Development in Industry, 1957: Performance and Financing*, made for the National Science Foundation by the Department of Commerce, Bureau of the Census, is the first in a series of annual surveys to be conducted in the United States by that Bureau for the Foundation (Surveys of Science Resources Series. Pp. xiv + 119. Washington, D.C.: Government Printing Office, 1960. 65 cents). More than half the total 7,200 million dollars was for research and development in the aircraft and electrical equipment industries, and 52 per cent of such work was financed by the Federal Government; 241 million dollars was for basic research; 1,514 million dollars for applied research; and 5,401 million dollars for development. Of the total, 6,060 million dollars was expended by companies employing 5,000 or more persons. Chemical and allied products; stone, clay and glass pro-

ducts; petroleum refining and extraction; and primary metals financed 90 per cent or more of their research and development. The average cost per scientist and engineer in research and development was 34,300 dollars, varying from 20,200 dollars in food and kindred products to 42,300 dollars in aircraft, and from 20,400 dollars in companies with fewer than 1,000 employees to 37,400 dollars in companies with 5,000 or more. Wages and salaries averaged 51 per cent of research and development costs in 1957, ranging from 46 per cent in the electrical equipment industry to 67 per cent in the paper and allied products. Company-financed research and development (excluding that federally financed) averaged 1·6 per cent of net sales, and varied widely from less than 1 per cent in food and kindred products, paper and allied products, petroleum refining and extraction, and primary metals, to 6 per cent in scientific and mechanical measuring instruments; but including federally financed research and development the average was 3·3 per cent of net sales.

Employment of Librarians in the United Kingdom

THE recent annual report of the Manchester Public Libraries Committee referred to difficulties which were being experienced through the rapid turnover of staff and the progressive weakening of professional experience. The Library Association has been increasingly disturbed in recent years by the extent to which it has been necessary for public libraries to fill professional posts with partly qualified staff: whereas in 1957 only 16 per cent of administrative, professional and technical posts were occupied by unqualified staff, in 1960 it had risen to 27 per cent, and at the end of 1960, out of a total of 2,738 posts, 277 were vacant and 998 occupied by unqualified staff. This situation led the Library Association to seek information from 484 public libraries regarding their turnover of staff since January 1959, and replies received from 352 showed that 47 per cent of qualified staff leaving were lost to the service, only 56 per cent of the vacated posts were filled by qualified librarians and 10 per cent remained unfilled. There was a loss of 299 qualified librarians to other types of library and public employment.

These and other figures were quoted by Mr. A. Fitch, who raised the subject in an adjournment debate in the House of Commons on March 1 and referred to the estimate in the Roberts report that an increase of more than 2,000 qualified librarians in the public library service was required for an efficient service. He suggested that the shortage was to be attributed to the unsatisfactory salary scales, and that the recommendations of the Roberts Committee were being ignored by the library authorities. In replying on the debate, the Parliamentary Secretary to the Ministry of Education, Mr. K. Thompson, referred to the Minister's announcement on December 15 that it was the Government's intention that the Minister of Education should have general responsibility for the supervision of the public library service, and that after certain preliminary work had been completed legislation would be laid before the House. Mr. Thompson said that this preparatory work was actively proceeding and broad measures of agreement already existed on many of the problems. Whatever new duties might be laid on the Minister of Education, the library service would continue to be an essentially local authority service, with Ministerial intervention limited to the minimum consistent with ensuring the maintenance of uniformly high standards. The Government accepted the view of the Roberts Committee that standards of staffing should be high both in numbers and quality, but he insisted that salary scales and conditions of service must be negotiated between librarians and the authorities employing them and that the Minister had neither the power nor the duty to intervene. As regards wastage of staff, Mr. Thompson thought that in so far as this applies to staffing of libraries elsewhere it might be to the advantage of the library system as a whole and encourage inter-library co-operation.

Abstracts of Human Developmental Biology

Excerpta Medica has issued the first of a new monthly journal called *Abstracts of Human Developmental Biology* (1, No. 1; January 1961. Pp. 52. Abstracts No. 1–164. Published monthly. Amsterdam and London: Excerpta Medica Foundation, 1961. Subscription rate: £6 18*s*. per annum). The new journal covers the experimental and clinical aspects of normal and pathological human development, including fundamental aspects of general morphogenesis, of reproduction, of transplantation in postnatal life, and of pathological growth, as compared with normal development, differentiation and growth. The new journal, developed and published with the aid of a grant from the United States Public Health Service, will contain approximately 2,000 abstracts a year, and will include classified subject and author indexes. It will thereby contribute to collaboration between specialists in the field of obstetrics, surgery, paediatrics, social medicine, embryology, experimental teratology, genetics and immunology, by integrating information in all these disciplines so far as they concern the problems of normal and abnormal development, reproduction and transplantation.

Florida State Museum, University of Florida

THE Director's report for 1959–60 of the Florida State Museum, University of Florida, has recently become available (Pp. 19+2 plates. Gainesville, Fla.: University of Florida, Florida State Museum, 1960). This Museum, which serves the people of the State and the University community in particular, is much hampered by lack of space and also of staff. The Department of Exhibits operates in three distinct areas of service. New exhibits are produced and loan exhibits are scheduled for the halls in the headquarters museum, a major reconditioning operation to modernize the archaic exhibits in the Seagle Building is in progress, and its influence also works in the aptly named 'out-reach exhibits'. Among the special exhibits are "You and the Atom", which illustrates the basic principles of nuclear energy and peace-time applications in medicine, agriculture and industry.

Control of Red Scale in Citrus Fruit

RED scale is the most important insect pest of citrus fruit in Australia. Standard control methods involving oil sprays or fumigation with hydrocyanic acid are by no means satisfactory. Mr. B. H. Martin and Dr. R. F. Black, of the Griffith Irrigation Research Station, have studied the possibilities of heat treatment as a control measure. An essential feature of any such treatment is that it will kill the scale without damaging the tree or the fruit. The experiments

have been described in a recent issue of *Rural Research* (23, Sept. 1960). The technique used in the experiments consisted of heating scale-infested fruit in ovens at different temperatures and for different periods of time. The effect of heat on fruit was therefore examined but not the possible damage to trees. It was found that all the scales could be killed by a temperature of 70° C. maintained for 1 hr. This treatment, however, resulted in severe damage to the fruit. Equally effective 'kills' were obtained at a temperature of 50° C. kept up for 2½ hr. During this treatment the fruit was not damaged. Increasing the humidity during the 50° C. treatment did not alter the time necessary to kill the scale, and it is apparent that high temperature rather than dehydration was the direct cause of death. Further experiments have involved treating the trees themselves. The technique used was to enclose the trees in plastic sheets and utilize solar energy as the heat supply. Some difficulty has been experienced in maintaining temperatures at a safe level.

Congelifraction of Clays

The clay fraction of soils is customarily regarded as a product chiefly of chemical rather than mechanical processes. While chemical alteration is undoubtedly necessary to produce clay minerals in the first place from the components of crystalline rocks, it now seems that at least one form of mechanical weathering, namely, congelifraction, can be effective in reducing particle size well down into the clay grade in unconsolidated sediments. I. C. McDowall (*N.Z. J. Geol. and Geophys.*, 3, 337; 1960) has investigated the effects of alternate freezing and thawing on water-saturated samples of kaolinite, vermiculite, halloysite, and weathered clays containing illite and halloysite. The particle size distributions were determined at intervals by the Andreason pipette method, and it was found that appreciable reduction of particle size occurred during 800 cycles. The process appears to operate on particles at least as small as 1μ, and is most rapid in those minerals where the bonding between the basal planes of component sheets of the clay mineral is weakest. In cold climates the process might therefore be an important one in the formation of plastic clays, perhaps particularly of fireclays.

Navigation down the Centuries

A short review of navigational methods from the earliest times to the present, with special emphasis on the newest techniques with Earth satellites, is made in a 24-page publication of the Coast and Geodetic Survey of the United States Department of Commerce, entitled *Use of Artificial Satellites for Navigation and Oceanographic Surveys*, by Paul D. Thomas (Technical Bulletin No. 12. Pp. i+24. Washington, D.C.: Government Printing Office, 1960. 25 cents). The first half of the publication is a historical review, beginning with Hipparchus and Ptolemy, touching on Galileo, the French expeditions of the seventeenth and eighteenth centuries, and the development of the marine chronometer, before coming to radar systems, *Loran* and inertia navigation. The second half of the paper is devoted to Earth satellites. Here the main interest is in the transit system of satellites, already in action on an experimental basis, from which position can be obtained by interpretation of the Doppler shifts of the radio-waves received from the satellites.

Use of Superconductors for producing Strong Magnetic Fields

The production of strong magnetic fields by the use of superconductivity phenomena would appear to be considerably advanced by the development of a new superconducting compound of niobium and tin, Nb_3Sn, by the Bell Telephone Laboratories. The distinctive property of this material is its ability to retain superconducting properties even when exposed to fields of about 90,000 oersteds, and possibly even higher, while carrying currents of approximately 100,000 amp. cm.$^{-2}$ (see Kunzler *et al.*, *Phys. Rev. Letters* for February 1, 1961). The new material is very brittle, but by packing a narrow niobium tube with appropriate quantities of powdered niobium and tin, sealing the ends of the tube with niobium plugs, reducing the tube by swaging and drawing it, a wire of 0·015 in. diameter which can be wound into a solenoid coil is formed. On heating the wire to about 1,000° C., the inner materials react to form Nb_3Sn, and the necessary superconducting properties are developed. Owing to mechanical difficulties associated with the substance at high fields, a large solenoid of the material has not yet been wound, but it is anticipated that these difficulties will be overcome within the next twelve months.

The Iron and Steel Institute, the Institute of Metals and the Institution of Metallurgists

The Councils of the Iron and Steel Institute, the Institute of Metals and the Institution of Metallurgists have recently examined the problems of closer co-operation between these three societies. After careful consideration they have concluded that amalgamation of the three societies would not be practicable within the foreseeable future. The three Councils, therefore, decided to set up a permanent Joint Consultative Committee of the three societies, which will meet regularly and advise their Councils on all activities and questions of common interest.

Lederle Medical Student Research Fellowships

The Lederle Laboratories Division of the American Cyanamid Co. announces that it is making available fellowships to medical schools throughout the United States and Canada for the year 1961. These fellowships, in amounts not exceeding 600 dollars a year for any one individual, are intended to relieve in part the financial burden of students who desire to devote their summer vacations to research in the preclinical departments. Students who apply for Lederle Medical Student Research Fellowships must have the consent of the faculty member under whose supervision their research is to be conducted. Further information can be obtained from the Lederle Laboratories, American Cyanamid Co., Pearl River, New York.

Carlsberg–Wellcome Travelling Research Fellowships, 1961–62

Applications are invited for the Carlsberg-Wellcome travelling research fellowships, 1961–62, the object of which is to encourage co-operation, on an exchange basis, between Danish and British research workers in any branch of the natural sciences which has a bearing on human and animal medicine. One fellowship annually is awarded to a candidate from the United Kingdom for a year's work in Denmark and one annually to a Danish

candidate for a year's work in the United Kingdom. The stipend may range from £900 to £1,300 per annum (or the equivalent sums in Danish kroner), travelling and some incidental expenses being provided in addition. The tenure of the awards will start in September 1961. Further information can be obtained from the Assistant Scientific Secretary of the Wellcome Trust, 52 Queen Anne Street, London, W.1. Applications must be submitted before April 30.

Medical Research Fund for Australia and New Zealand

A FUND has been established by the Life Offices' Association for Australasia to promote and assist scientific and medical research. For the present it is intended to use the resources of the Fund to stimulate and support research in the field of cardiovascular function and disease. Grants from the Fund will be made as follows: *grants in aid*, which will be given to non-profit institutions with basic facilities and qualified personnel for medical research, and will range from £1,000 to £3,000; *research fellowships*, which will be open to medical graduates or those with equivalent scientific training, and will fall into two categories: (*a*) fellowships taken up in Australia and New Zealand for a period of one year (£2,200–£2,500 p.a.); (*b*) travelling fellowships, for a period of two years abroad, followed by a third year in an approved institution in Australia or New Zealand (£2,500–£3,000 p.a.). Further information and application forms can be obtained from Dr. H. M. Whyte, Life Insurance Medical Research Fund of Australia and New Zealand, 87 Pitt Street, Sydney. Applications for all awards must be received by July 1.

André Mayer Food and Agriculture Fellowships

THE Food and Agriculture Organization of the United Nations has announced that it will offer ten or twelve André Mayer fellowships in 1961. These awards will be made under the following two groups: *research*, for unusually promising persons with research experience behind them who would offer a guarantee for the carrying out of independent research work; *research training*, for younger and promising individuals who have demonstrated an inclination towards, and an aptitude for, research work. Due consideration will be given to geographical distribution, to ensure that a proportion of the awards is given to candidates from countries where research facilities are not highly developed and the need for trained research workers is great. The subjects presented must be in relation to the Organization's activities, which comprise: land and water development, plant production and protection, animal production and health, rural institutions and services, fisheries, forestry and forest products, nutrition (non-medical), atomic energy in food and agriculture, agricultural economics (commodities, statistics, economic analysis). Further information can be obtained from the National Committee for the United Kingdom of the Food and Agriculture Organization, Ministry of Agriculture, Fisheries and Food, Whitehall Place (East Block), London, S.W.1. The closing date for application is April 30.

University News: Birmingham

THE following appointments have been announced: Dr. M. Z. Atassi, to be Research Fellow in the Department of Chemistry; Dr. B. Cockayne, at present Research Fellow in the Department of Physical Metallurgy, to be lecturer in the Department of Physical Metallurgy; M. J. Hamlin, to be lecturer in the Department of Civil Engineering; Dr. D. A. Smith, to be lecturer in the Department of Microbiology. The title of reader in neuropharmacology has been conferred on Dr. P. B. Bradley, senior lecturer in electrophysiology in the Department of Experimental Psychiatry.

Edinburgh

DR. DUGALD GARDNER has been appointed as senior lecturer in pathology. Dr. R. C. Dorf has been appointed as a lecturer in electrical engineering, and T. Astarabadi as a lecturer in physiology.

Announcements

DR. C. J. JACKSON has been appointed chairman of the Water Pollution Research Board for the period April 1, 1961–March 31, 1966, in succession to Dr. F. H. Garner, who completed his term of office on March 31. Dr. Jackson is in charge of all water pollution problems for the Distillers Co., Ltd., both on the potable and industrial sides.

PROF. J. A. GOEDKOOP has been appointed managing director for research at the Reactor Centrum Nederland, where he was previously in charge of the Physics Department. Prof. Goedkoop has been at the Centrum since its foundation in 1955. For some years he directed the physics research at the Norwegian–Netherlands Joint Establishment for Nuclear Energy Research (JENER) at Kjeller, Norway. He is also a part-time professor at the Leyden State University.

A JOINT meeting of the Challenger Society and representatives from the Marine Laboratories (Development Commissioners' Scheme) will be held at the Royal Dublin Society, Ballsbridge, Dublin, during May 24–25. Further information can be obtained from the Challenger Society, c/o Dr. A. E. J. Went, Fisheries Division, Department of Lands, 3 Cathal Brugha Street, Dublin.

THE Nutrition Society (Scottish Group) is holding a symposium on "The Relative Values of Animal and Vegetable Fats in Nutrition" at the Physiology Lecture Theatre, Queen's College, Dundee, on April 22. Further information can be obtained from the honorary secretary of the Scottish Group of the Nutrition Society, Dr. J. Davidson, Rowett Research Institute, Bucksburn, Aberdeen.

THE annual general meeting of the Oil and Fats Group of the Society of Chemical Industry is to be held at Liverpool on April 25. It will include an address by Dr. A. T. James, of the National Institute for Medical Research, on "Applications of Gas Chromatography to Some Medical Problems". Further information can be obtained from Dr. H. Jasperson, c/o J. Bibby and Sons, Ltd., Research Department, King Edward Street, Liverpool 3.

ERRATA. Two mistakes in the list of new Fellows of the Royal Society of Edinburgh (*Nature*, March 18, p. 876) have been pointed out: Dr. K. B. Fraser is senior lecturer in bacteriology (not botany) in the University of Aberdeen; and the name of Dr. C. H. Gimingham, senior lecturer in botany in the University of Aberdeen, was omitted.

AFFORESTATION OF UPLAND HEATHS

FORESTRY Commission *Bulletin* No. 32 purports to deal with what are termed 'upland heaths', which are said to occur along the eastern side of Scotland and northern England, and to be distinguishable from lowland heaths formed farther south*. They are distinct from the peat-covered lands in the western parts of the country, which were the subject of an earlier *Bulletin*—No. 22—by the same author, entitled: *Experiments in Tree Planting on Peat* (H.M. Stationery Office, 1954). It reviews experimental work carried out over a period of thirty-six years during 1921–57, and records all that was attempted by the Forestry Commission, "with an assessment of the results so far".

The last phrase gives the clue to one of the major obstacles which research workers encounter when they undertake experiments on a field scale with the view of improving sylvicultural and management techniques. It is not possible to bring such experiments to a conclusion within less than a century, especially when they are conducted on such infertile sites as the degraded forest soils of early centuries, now covered with ericaceous vegetation, which go by the name of heaths. These soils, it is now generally accepted, have arisen as a result of forest destruction in the past, and of repeated burnings and heavy grazing which have killed off the woody vegetation. Whatever the original composition and structure of the former forest soils may have been, these have been lost, and the problem of the restoration of the soils to their original relatively fertile state is immense.

Since these heath sites are almost entirely unproductive from an agricultural point of view and should never have been deforested, it is only right and proper that they should, if possible, be reforested; and it is not to be wondered at that the Forestry Commission has acquired, and continues to acquire, extensive areas of such 'marginal' land, and rightly so.

The length of time which this kind of study requires has another important effect, namely, that no one individual can commence the work and bring it to completion in his life-time. If he could, it is more than likely that by the time he reached the end of his span he would have forgotten what the conditions were when he began his experiments. In these experiments no fewer than eight senior officers and three junior officers are credited with having played an important part, not counting the author himself. The difficulty of seeing the work as a whole and in its proper perspective is increased by so many changes over such a relatively short time. Another embarrassment to the forest research worker is that, no sooner do his experiments begin to indicate apparent success, than the inexperienced general practitioner draws his own conclusions and immediately applies a new method before it has been tested thoroughly. The older forester is more wary.

This particular experimental project was begun in 1921, and the major experimental areas were at first concentrated in six forests, four in Scotland, and two in the north of England. Later on experimental work was extended on a less-intensive scale to other forests. The kinds of experiment attempted were many, too many to be handled adequately, but the principal projects dealt with were: methods of ground preparation; various kinds of ploughing; trials of species; use of manures at time of planting; and later, use of nurse trees and mixtures of species, besides a variety of miscellaneous procedures. The result of all this was an accumulation of a vast amount of mensurational and other data. The earlier experiments were somewhat crudely set out, but later widespread use was made of approved methods of layout and of statistical methods of handling the results. Mr. Zehetmayr is to be congratulated on having presented his information in a very readable form and on treating the problem with admirable judgment. The *Bulletin* contains a mass of information on method and species. The conclusions are presented in an excellent summary, and it must be said that they do not differ essentially from those reached by tree-planters in Britain long before the Forestry Commission was born, except in respect of the newer species. The early work of the Commission was bedevilled by an idea that spruces, because they are highly productive on suitable sites, should be grown everywhere, even on upland heaths. Another herring drawn across the trail was the idea, imposed from above, that the deeper one ploughed the better. The unjustifiable opinion was held, and often still is, that the trees could be compelled to extend their roots down into the execrable material on which the heath grows. Fortunately, the experiments indicate that it is more effective to prepare the surface-layer than to bring up a mass of inert mineral matter, containing a small proportion of soluble minerals, which in time return to the depths whence they came. On sites with richer sub-soils nearer the surface, deep ploughing seems to be more useful—at least for a time. There is an undoubted stimulus to tree growth on ploughed land due to improved mineralization of the organic matter, but how long it continues remains to be seen. It is claimed that ploughing has extended the area on which planting is economically possible and has improved growth on moderately fertile areas. The possibility of future troubles is not overlooked.

It is interesting to find that the author believes, and rightly, that for these upland heaths, Scots pine will remain the most important tree. Corsican pine and Japanese larch—more exacting trees—do not seem to be suitable substitutes, but the shore or lodgepole pine from North America is a promising introduction.

This publication, like certain others produced by the Forestry Commission, can be criticized on a number of points. In the first place, some attempt should have been made to use the classification of heath-types dealt with in Chapter 1, and to treat them by classes. For example, upland heaths on the Red Sandstone areas of Scotland are radically different from those on the Jurassic of the Cleveland

* Forestry Commission. *Bulletin* No. 32: *Afforestation of Upland Heaths*. By J. W. L. Zehetmayr. Pp. v+145+34 plates. (London, H.M. Stationery Office, 1960.) 17s. 6d. net.

Hills of Yorkshire. The so-called 'pans' differ markedly, depending on the looseness of texture of the underlying glacial deposits. The essential difference between the upland heaths of Britain and the continental heaths of Denmark, Germany and the Netherlands is clearly explained in Chapter 2. While such differences may justify the adoption of a completely independent attitude in Britain they do not excuse the neglect of the important work done in France by Duchaufour on heath soils or by Scandinavian, other than Danish, workers. Nor is the ignoring of the heath afforestation carried out in Great Britain long before the Forestry Commission was constituted excusable. Whether Continental heaths have "a longer history of systematic afforestation than our own" depends on what is meant by 'systematic'. The skilled tree-planters of Britain, in the eighteenth and nineteenth centuries, with a life-time of experience behind them, came to very definite conclusions about suitability of species, the value of ploughing and other techniques, and in forestry a life-time of experience in one locality may compensate for lack of intensive experiment at heavy cost, and the use of statistical methods to support the very obvious results which the intelligent observer sees and records in his memory, if not in his note-book. This is possibly rank heresy, but when one is confronted with the meagre conclusions from thirty-six years of intensive experiments, involving a multitude of measurements and calculations, one may be permitted to wonder if the capacity of the human brain is not nowadays underestimated.

Mr. Zehetmayr deserves great credit for the immense pains which he has taken to range over space, at least, and make himself conversant with heath-land planting. He has dealt with the enormous accumulation of experimental data in a masterly manner and makes good use of figures and illustrations. As a result of his breadth of vision, his conclusions are sound and are devoid of the exaggeration and puerile propaganda which sometimes detract from the value of forestry technical publications. He also places the important work so far achieved in its correct perspective historically, although perhaps his glance has been directed too far ahead and not enough backwards. Much more intensive soil research, as opposed to more-or-less empirical experimentation, is now required if further real progress is to be made. Meanwhile, adopting the same cautious attitude as the author, foresters and tree-planters would do well to be conservative, rather than rash, in their attitude, and to eschew the high-living tree species in favour of the abstemious. They should read and apply the lessons so far learned with discrimination, paying special heed to variation in locality conditions. In particular, deep ploughing on a large scale should not be used indiscriminately; it is often not only unnecessary but also harmful, especially in its effect on the drainage of catchment areas. The dweller in the lowlands is often unaware of what is happening in the uplands in these days. The excellence of Mr. Zehetmayr's interpretation will give great satisfaction to all who have participated in this project.

M. L. Anderson

REORGANIZATION OF BRITISH NATIONALIZED TRANSPORT UNDERTAKINGS

NEITHER in the White Paper on the *Reorganization of the Nationalized Transport Undertakings** nor in the debate on January 30, in which the Minister of Transport, Mr. E. Marples, asked the House of Commons to take note of the proposals for reorganization, was any reference made to the importance of research or of a sufficiency of scientific and technical man-power in Britain. Furthermore, the organization chart appended to the White Paper gives no indication as to how the organization for research is affected. The White Paper puts as the Government's aim undertakings soundly based on organization and finance, providing efficient services and giving a good livelihood and worth-while jobs to those who work in them. It directs attention to the fact that the railways are a vital basic industry, employing 500,000 people and representing an investment of nearly £1,600 million, still growing by more than £100 million each year. A railway system of the right size is regarded as an essential element in the British transport network, and will remain so for as long as can be foreseen, but the White Paper does not discuss the vital question: What is the right size? The related question of congestion on the roads is ignored, and while the idea of the railways as a social service is rejected, the question of uneconomic services such as branch lines is reserved for further consideration; it is merely stated that for the time being railway losses on any such services will be covered by the contributions proposed from public funds.

This point was stressed during the debate by Sir Toby Low, chairman of the Select Committee on Nationalized Industries, who, while agreeing that, in the main, the Government's proposals were in line with the recommendations of the Select Committee, was doubtful whether full control over the finances of the railway system could be achieved until the problem of dealing with uneconomical services had been decided. Sir Toby insisted that we could find out why losses are incurred, and he urged in conclusion that the real problem was not money but morale. Success can only be achieved by the vigorous use of every instrument of management and of science. Apart, however, from the Minister's announcement that the Government had decided that the main-line electrification scheme from Euston to Crewe, Manchester and Liverpool should go ahead as quickly as was consistent with the best use of the available resources, nothing in the debate clarified the uncertainty about the progress of modernization or the future of the uneconomical services. The Joint Parliamentary Secretary to the Ministry of Transport, Mr. John Hay, did not accept Sir Toby Low's challenge, and only stated that in 1960 the Treasury provided £160 million and in 1961 £140 million for investment in the railway industry, as a result of which more than seventy major projects had already

* Ministry of Transport. *Reorganization of the Nationalized Transport Undertakings*. Pp. 14. (Cmnd. 1248.) (London: H.M. Stationery Office, 1960.) 1*s.* net.

been approved. Earlier, Mr. Marples had said that he would have to keep the electrification scheme (which is to cost £175 million) under close review to see how the estimates of cost and the rate of working compared, but that this did not necessarily mean that no other major main-line electrification scheme would be approved.

The White Paper states that the main conclusions reached by the Government are that the activities of the British Transport Commission, as at present constituted, are so large and diverse that it is virtually impossible to run them effectively as a single undertaking. There has been confusion in judging between what is economically right and what is socially desirable, and the commercial capability of the railways is circumscribed by outmoded statutory obligations and restrictions on their trading operations. Mounting deficits (now totalling £500 million), the size of capital debt in relation to the earning capacity of the assets and the increasing burden of interest (already some £75 million alone) as modernization proceeds, present a situation detrimental to the morale of management and workers, to financial control and to hope of recovery. Accordingly, the Government has decided to replace the British Transport Commission and the existing organization by a new structure designed to overcome the main defects and disadvantages of the present organization, to reconstruct the finances of the Commission, and of the railways in particular, and to give the various undertakings the maximum practicable freedom of operation in their commercial affairs.

In the new structure, each of the main activities of the present Transport Commission—railways, London Transport, docks, inland waterways—will be managed by a separate Board holding its own assets and responsible for its own capital debt. The British Railway Board will be responsible for running the railways as an effective national system, but will perform only those central functions which are essential to the running of the railways as a single entity, including policies for safety, training and research, and the determination of the future size and shape of the railway system; all other functions will be the responsibility of the Regional Railway Boards. Co-ordination of policy between the new Boards for railways, London Transport, docks and inland waterways will be the responsibility of the Minister of Transport, assisted by a new Nationalized Transport Advisory Council. His special duties in respect of safety, training and research will continue. As regards finance, the Government proposes to write off £400 million of the £2,000 million capital liabilities of the British Transport Commission, and when £400 million of the remainder has been transferred to the appropriate new Boards, to place in suspense £800 million of the £1,200 million still remaining against the railways so that it carries neither fixed interest nor fixed repayment obligations. The amount and treatment of this suspense account will be subject to review from time to time in the light of developments. It is expected that the railways will eliminate their present operating loss of £60 million a year. The importance of efficient operation and the most economic use of man-power is also emphasized, and the railways are to be freed from statutory control over their charges except for fares in the London Passenger Transport Area.

THE AGADIR EARTHQUAKE

FOLLOWING a detailed local study of the Agadir earthquake of February 29, 1960, P. Erimesco has published the conclusions in the *Bulletin de L'Institut des Pêches Maritimes du Maroc* (No. 5; September 1960). The author states that earth tremors in Morocco are not infrequent, although they very seldom attain an intensity of more than 5 on the Mercalli scale. In the list given in the text for the period from May 31, 1934, to October 28, 1951, eighteen earthquakes have intensity 4 or greater. Two of these were of intensity 7, and four had maximum intensity 6. The town of Agadir is situated at the intersection of two lines of marked seismicity. One line follows the region between the High Atlas Mountains and the Anti Atlas. The other crosses the High Atlas Range and continues to the coast of the Mediterranean.

During the night of February 29, 1960, after two slight tremors, an earthquake of unusually high intensity for the region (IX–X) devastated the town of Agadir (initial time 23h. 40m. 12s. G.M.T.). According to the map produced by the author, showing isoseismal lines for the shock, the small region of greatest intensity inside isoseismal X lies just on shore. The local estimate of depth of focus lies between 3 and 7 km., and of the magnitude of the shock 5·75 to 6. According to Vít Kárník, of Czechoslovakia, the average formula connecting magnitude (M), intensity (I_0) at epicentre, and depth of focus (h) for European shocks is:

$$M = 0{\cdot}67\, I_0 + 1{\cdot}7 \log h - 1{\cdot}4$$

For $I_0 = 10$ and $h = 5$ km. this gives $M = 6{\cdot}48$, which is somewhat higher than the local estimate.

Whole areas of the town, situated near the port and along the shore, suffered very heavy damage, and in certain places total destruction. A squadron of the French Navy, rushing help to the stricken town, registered on the way two hitherto unknown depths of 15 m. and 10 m. where charts indicated 900 m. and 90 m., respectively. To check these soundings taken visually, two vessels of the Navy made, the next day, a systematic search. At the places in question, no echo at all could be obtained.

At the moment of the earthquake, the trawler *Rolando*, heading south-west, passed approximately the place where later the 10-m. depth was observed. The crew reported that they felt three shocks, as if their boat had hit a solid object. The sea became very choppy for several minutes and a glow appeared in the sea, shifting rapidly towards the town of Agadir. Shortly afterwards they saw the lights in the town going out.

In spite of near-by badly damaged buildings, the quay walls were found practically without serious deformation, with the exception of two short oil wharfs. The slip for the fishing boats had not suffered at all. The tide gauge at the time of the earthquake registered the choppy sea in the harbour usual during that time of the year on account of wind

from the south-west, but no exceptional waves. To investigate changes on the sea-floor consequent on the earthquake, P. Erimesco with his assistants examined the effects in the harbour of Agadir and along the shore. In the Bay of Agadir, systematic soundings were made and samples were taken from the bottom at the place where the 10-m. depth was observed. The soundings could not detect any variation from the depths marked on the charts; and the bottom samples at the place where volcanic activity had been suspected contained living annelids in perfect condition.

Thus, contrary to local reports at the time of the earthquake, we have evidence of a most unusual occurrence, namely, an area of intense earthquake destruction practically on the shore line, and virtually no effect at sea—neither the sea bottom nor the sea waves—except perhaps the rising of a shoal of zooplankton and a cloud of mud in the sea.

P. Erimesco seeks to explain this by reference to the local geology. He states that from the geological structure of the region it can be inferred that the almost vertical Cretaceous and Tertiary strata near the coast are dipping steeply underneath the sea bed. The covering Quaternary and Recent layers are subhorizontal or horizontal and, in their upper part, not yet compacted, and are saturated with water. Earthquake waves in the Cretaceous, or in the underlying folded Palæozoic strata, are thus propagated mainly in the solid rock, and, by total reflexion, literally channelled towards the outcrop of these strata on the coast. In the unconsolidated and water-saturated material at the bottom of the sea, the energy of the shock waves is largely dissipated in the material, and only locally can a fraction of the energy released by the earthquake reach the surface of the sea. The trawler *Rolando* might have been at just such a place.

E. Tillotson

SEDIMENTATION IN OROGENIC BELTS

A LITTLE more than twelve years ago, Kuenen and Migliorini set out the evidence which had led them independently to the fruitful idea that certain marine sediments may be re-deposited in deeper water, possibly many hundreds of kilometres distant from their temporary resting places, by the action of turbidity currents. The speakers at the symposium "Some Aspects of Sedimentation in Orogenic Belts", held by the Geological Society at the Royal Institution on February 8, had a wide field over which to range, but it was remarkable how much of the work described stemmed directly or indirectly from this concept.

In opening the symposium the president, Prof. S. E. Hollingworth, reminded the audience how present-day views on re-sedimentation could be traced back to the work of Bailey and O. T. Jones in the 'thirties. The principal speakers in the first session dealt with work in the Alps (Prof. R. Trümpy), the Polish Carpathians (Prof. M. Ksiazkiewicz) and the Himalayas (Prof. W. D. Gill). In the second session Prof. A. Wood spoke on the Lower Palæozoic of Wales, Prof. Scott Simpson on the Upper Palæozoic of south-west England, Prof. R. M. Shackleton on the Dalradians and Prof. Gill on a Carboniferous basin in Ireland. Discussion followed in each session. A summary account will appear in the *Proceedings of the Geological Society*, and rather than duplicate this summary I propose to consider in a more general way some of the topics which came up during the meeting.

Prof. Trümpy, who has recently provided a welcome account of alpine sedimentation[1], opened with a warning note on the use of the term 'flysch'[1,2], which, he said, describes a facies group and not a rock. Flysch, he continued, is marine, thick (> several 100 m.), and essentially composed of sandstones and shales in proportions varying from about 5 : 1 to 1 : 10. Individual beds can be followed for long distances, and flysch is in general monotonous. The shales are silty and the sandstones are often felspathic and generally micaceous. True greywackes are rare. Breccias and conglomerates, though quite subordinate, are typical of many flysch deposits. Prof. Trümpy pointed out that graded calcarenites are not called flysch in the Alps.

The structural setting in which flysch appears was discussed by several speakers. Prof. M. Ksiazkiewicz[3-5] showed how flysch was deposited in various parts of the Carpathian basin at different stages of its evolution. Thus in the outer zone folded in the Lower Miocene the flysch formed from the end of the Jurassic without interruption until the Oligocene. In the middle zone flysch did not appear until the Lower Senonian shortly before the pre-Maestrichtian orogenic stage, while in the inner zone flysch rests on rocks folded at the end of the Cretaceous. In the Alps, Prof. Trümpy suggested, flysch marked a stage when tension gave way to compression. Prof. Scott Simpson made the point that flysch is not confined to fold belts, while Prof. Gill gave a striking account of a comparatively small and shallow epicontinental basin in the Visean of County Dublin, in which slides and turbidity currents had developed in a non-orogenic setting.

The difficult question of the depth of water overlying flysch deposits was approached in several ways. Prof. Trümpy placed the fossils of the Alpine flysch in three ecological groups; an authochthonous fauna of fossils such as fucoids and helminthoids derived from soft-bodied animals indicating deep water beyond the penetration of light; a transported benthonic fauna including the larger foraminifera, calcareous algæ and bryozoa, preserved in graded sandstone beds; and thirdly, pelagic microfossils such as globigerinids and radiolaria. Dr. J. F. M. de Raaf reported that foraminifera similar to or identical with recent deeper water species had been found in pelitic rocks above individual turbidites from flysch deposits in Switzerland, France, Spain, Italy and Greece. Profs. Ksiazkiewicz, Scott Simpson and Trümpy gave evidence which indicated that deep-water conditions were established before deposition of flysch began. Where shallow-water sediments were followed by flysch an intervening group of deep-water sediments was often present. In this connexion an interesting parallel emerged

between a succession from south-west England in which, during the Variscan orogeny, Upper Devonian shallow water sediments were followed first by deep-water shales, cherts and limestones and then by Namurian flysch, and certain Alpine successions in which shallow-water platform deposits were covered first by shales and fine-grained limestones with a purely pelagic microfauna and then by flysch. In both regions the arrival of flysch was heralded by a phase when sedimentation did not keep pace with downwarping. In contrast, Gill attributed the lack of flysch over the greater part of the Himalayas to the building up of land masses in Middle and Upper Eocene time.

Prof. A. Wood[10–12] described some of the variations in lithology shown by the Aberystwyth grits which he employed to demonstrate the changes which could occur in turbidites as they are followed down-current. He discussed the behaviour of the associated mudstone and suggested that under certain conditions muds were stripped off and added to the turbid suspension passing overhead. Detailed work of this nature and of the kind carried out by Radomski in the Alps is beginning to establish the relationship between changes in facies and the several environments which might be expected in a single basin and seems likely to improve our understanding of the mechanism of turbidity currents.

Later in the discussion Mr. W. A. Cummins suggested that the finer-grained chloritic fraction of some greywackes might in part be a secondary feature. The apparent lack of contemporary greywackes might be explained in this way. Dr. J. G. Ramsay and Dr. J. L. Knill mentioned the difficulties which may arise in establishing the original attitudes of deformed sedimentary structures. Although, as Prof. Wood pointed out, account is usually taken of the effects of plunging structures, so far as I am aware the effects of shear folding in which the angle between a sedimentary structure and the fold axis alters during folding have not been considered. The results presented by Ramsay[16] on this question should allow observations to be made in rocks deformed in this way.

Prof. Shackleton summarized present knowledge of Dalradian current directions[13–15], and commented on the distribution of the various Dalradian sediments, some of which remain remarkably consistent from western Ireland to north-east Scotland. Dr. B. Sturt described variations within the Dalradian of Perthshire and Prof. Shackleton mentioned the recent recognition of turbidites near the base of the Torridonian succession in Colonsay and Islay.

One of the most arresting features of the symposium was the sequence of maps presented by Prof. M. Ksiazkiewicz[3–9], which illustrated the results of measurements of current directions and facies analyses in the Polish part of the Carpathian flysch zone. This work, carried out by Prof. Ksiazkiewicz and S. Dzulynski, A. Slaczka, K. Zytko, W. Sikora, L. Koszarski and A. Radomski, has resulted in an elegant demonstration of the forms of the troughs and of the whereabouts and nature of the source areas. The varying directions taken by sediments were shown, and it was demonstrated, for example, that for a considerable time the axes of two neighbouring troughs sloped in opposite directions so that a northern trough was fed from the west and a southern from the east. These important results provide one of the most detailed accounts yet established of the movement of sediment within an evolving orogenic belt. I suggested, when summing up the day's discussion at the close of the meeting, that a synoptic account of comparable observations made in the British Palæozoic might be useful in bringing out what is known on this topic and what we need to find out in the future.

J. Sutton

Alps

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Carpathians

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ROCK ENGRAVINGS IN NORTHERN ITALY

SOME years before the First World War, l'Institut de Paléontologie Humaine was founded by Albert I, Prince of Monaco. Its various publications, not least the series of *Archives*, are of importance to every prehistorian. *Memoire* 31, entitled *Le Grande Roche de Naquane*, has maintained the standard. Rock engravings in the Val Camonica have long been known, especially as the result of the publications of G. Marro and R. Battaglia, but the time was ripe for this detailed study by M. Anati to appear. The carvings are found on rocks, many hundreds of them, up the valley for about 30 miles from near Boario to Edolo. A line drawn on a map from Bergamo to Bolzano will pass not far from the art complex. The author has attempted to separate these stylized drawings into three series: the first dating may be as old as the late Bronze Age, a second belonging to the Iron Age, and a third, which was still being made when Rome conquered northern Italy. A glance at the illustrations—there are no less than 52 full pages—reveals that there are depicted in a pecking technique animals, mainly domestic but including stags, human beings and various weapons and signs. The author decided not to select his illustrations from various sites in the complex, but to concentrate on a single rock surface. There is thus given a complete account of at least one locality from among the 600 or so which have been identified.

The Val Camonica is a long valley running up into the Alps, and it is probable that it has always been

a highway for people passing north and south. But why for 30 miles or so folk should have taken the trouble to cover rocks with stylized figures of animals, men and signs remains a mystery. It is easy to talk about ritual, but that does not answer the question. Why the ritual, and what was it? Similar rock art complexes are known, the most famous being those surrounding Monte Bego in the Ligurian Alps. While there is a certain similarity with those of the Val Camonica, there are also notable differences (the characteristic halberds of Monte Bego seem to be replaced by spears at Val Camonica and I do not remember any stags at Monte Bego), and in any event their connexion would rather be with the earlier series at the Val Camonica than with the later. At Monte Bego there is a thunderstorm almost daily at 3 p.m., due to the fact that it is the first high mountain inland from the hot Riviera coast. Local peasants, when asked what the weather will be, still say, "Let me see what the devil at Monte Bego is doing". One can imagine a reason for a ritual to take place from time to time at that mountain, but at Val Camonica such a reason would seem to be absent.

M. Anati is to be congratulated on producing a very useful and much-wanted work. Two criticisms must be added. No general index is supplied, and while there are references to a few works in the text, there is no bibliography. This is a real nuisance. French publications seldom carry any index: a policy to be questioned. In the present case, its absence is a great inconvenience. But these two defects must not be allowed to detract from the real value of M. Anati's excellent work. MILES BURKITT

HYPOTHERMIA AND THE EFFECTS OF COLD

THE modern investigation of hypothermia in man can be said to date from the work of Temple Fay and G. W. Smith, carried out during 1938–39. They cooled patients suffering from inoperable carcinoma, in the hope that the lowered temperature would affect the new growth more than the rest of the body. The clinical work was complemented by physiological work by D. B. Dill and W. H. Forbes on hypothermic patients.

During the Second World War hypothermia became important as a factor in survival at sea. Shipwreck survivors rescued after prolonged immersion frequently died because of lowered body temperature. Much work on the effects of body-cooling was thus stimulated, with emphasis on the treatment of hypothermia rather than on its clinical use. After the War, A. H. Hegnauer and his associates at Boston started a systematic investigation of the physiology of hypothermia in the dog, and about the same time W. G. Bigelow began his work at Toronto with the possibility of using hypothermia as a surgical tool.

Since 1950, the subject has advanced very rapidly, particularly as the surgical advantages of hypothermia have become apparent. Smith and Fay reduced body-temperature to between 30° C. and 32° C., and this was a hazardous procedure. For a long time it was found to be dangerous to lower the temperature to below 27° C. Now C. E. Drew and his colleagues cool their patients to below 15° C.

A recent issue of the *British Medical Bulletin* contains a fascinating collection of articles describing present investigations and how the techniques of hypothermia are being used in medicine and surgery (**17**, No. 1: January 1961: Hypothermia and the Effects of Cold. Pp. 1–78+xvii. London: British Council, 1961. 20s.). The articles include one by R. W. Salt on the resistance of poikilothermic animals to cold; hibernation in mammals by Dr. L. Harrison Matthews; local cooling in man by Dr. R. H. Fox; physiological effects of continued cold on animals and man by J. S. Hart; biochemical changes in exposure and acclimatization to cold environments by Dr. F. Depocas; the technique of induced hypothermia by Drs. I. K. R. McMillan and E. S. Machell; profound hypothermia in cardiac surgery by Dr. C. E. Drew. Other articles include accounts of experimental deep hypothermia and the circulation in hypothermia; metabolism in hypothermia; hormones in hypothermia; the central nervous system in hypothermia and the effect of temperature on the response to drugs; and sensitivity of hypothermic mammals to X-irradiation. Prof. A. S. Parkes has acted as co-ordinator of the whole series.

OPEN FORESTS IN TROPICAL AFRICA

PUBLICATION No. 52, issued by the Scientific Council for Africa South of the Sahara, contains selected papers presented at a conference of British, French, Belgian and Portuguese specialists held at Ndola, Northern Rhodesia, during November 17–23, 1959, and also the recommendations made and the conclusions reached*.

The conference considered the forestry problem of what is generally referred to as miombo woodland which, from Katanga (Congo) to Southern Rhodesia, covers some 670,000 square miles. The term miombo embraces several forest types ranging from dry high forest to savannah-woodland, but *Brachystegia* species are fairly typical of the tree canopy.

The sylviculture and management of the miombo woodland are made difficult by the large areas, the damage caused by fires, the low proportion of valuable species, the slow rate of growth, the long haulage distances, the limited uses of timbers which are not easy to work, slow to season, not durable and sometimes not possible to impregnate. *Pterocarpus angolensis* and the so-called Rhodesian teak, *Baikiaea plurijuga*, provide timber for constructional

* Scientific Council for Africa South of the Sahara. CSA/CCTA Publication No. 52. Pp. 126. (London: Bureau of Publications, CCTA/CSA Secretariat, Watergate House, York Buildings, W.C.2, 1960.)

work and for joinery, but most of the other timbers are used for fuel-wood, mine-wood and smelter poles in the Copper-belt.

As a result of burning experiments, covering a period of 26 years in Northern Rhodesia, there is ample evidence to confirm that late burning is most damaging to the vegetation and soil, complete protection is an unattainable ideal and that early burning is practical and beneficial by preventing damaging late fires, allowing the stocking of the trees to increase and not causing erosion.

Attempts are being made to resolve some of the problems of miombo woodland. There is a need for coniferous timber and it is being imported from Canada at great expense into the Copper-belt. In tropical and sub-tropical Africa mountain grassland is used largely for the planting of exotic conifers, but Northern Rhodesia has none. However, as a result of a well-planned, cautious programme, instituted some 20 years ago, tropical pines are being planted in suitable soils in miombo woodland.

A similar programme has been instituted in Katanga, but with eucalypts, and it is estimated that about one-hundredth of the area is required to yield the same amount of produce for the mines as can be obtained from miombo woodland.

It is good to note that not only were the problems affecting such large areas expressed at the Ndola Conference but also that steps have been put in train to exchange information between member Governments and to collect further information required for a fuller understanding of miombo woodland so that it may be put to better use.

AN A.C. MODULATED FLAME IONIZATION TRIODE

By J. MIDDLEHURST and B. KENNETT

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A CONVENTIONAL flame ionization detector operates as a diode, the ionization current of which is measured with a d.c. amplifier. The effects of drift and fluctuations in gain associated with such d.c. systems may be avoided by the use of an a.c. system. This may take several forms: the diode may be supplied with an a.c. voltage[1]; the d.c. current out of the diode may be converted to a.c.; the flow of gas to the jet may be modulated with an oscillating diaphragm; or the diode may be converted to a triode and used as an a.c. amplifier.

In a recent account[2] of the discussion at the third International Symposium on Gas Chromatography, L. Ongkiehong is reported to have suggested the insertion of a third electrode into the flame ionization detector. We have been investigating the properties of such a triode system for some time, and preliminary results are presented here. The triode detector has a large output and needs no further amplification of the signal, allowing a considerable reduction in associated equipment. By a suitable choice of operating conditions, the detector can have a linear or approximately logarithmic response.

The system used consists of a hypodermic needle (0·4 mm. int. diam.) as a jet and a 1-sq. cm. 48-mesh platinum gauze anode situated about 9 mm. above it. The third electrode, termed by us the grid, is a cylinder 4 mm. int. diam., 2·5 mm. long, wound from 0·5-mm. platinum wire, placed coaxially and approximately level with the jet.

Such a system may be treated in the same way as a triode valve. Fig. 1 shows typical characteristic curves taken at a constant flow of 10^{-8} gm./sec. of ethylene. Only characteristics involving positive anode potentials and currents are given, superimposed on which are two load lines *BC* and *DE*, each of slope 2×10^{9} ohms.

The way in which the system is used is as follows:

(1) Suitable values are chosen for the d.c. anode supply voltage and load resistor.

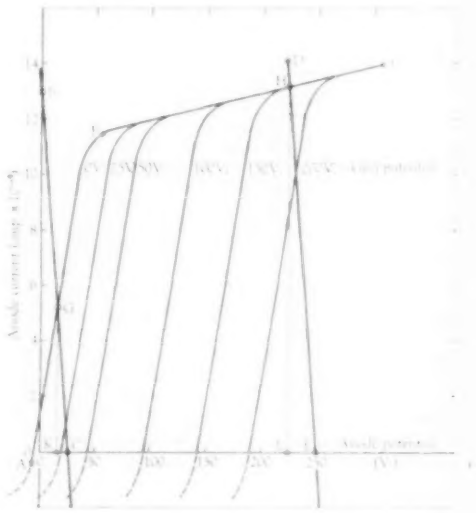

Fig. 1. Characteristic curves of flame ionization triode. Nitrogen flow, 50 ml./min.; hydrogen flow, 50 ml./min.; air flow, 800 ml./min.; ethylene flow, 10^{-8} gm./sec.; anode height, 9 mm.; grid height, 6 mm.

(2) A sinoidal voltage having a peak-to-peak value equal to the anode supply voltage is applied to the grid.

(3) A d.c. voltage equal to half the anode supply voltage is also applied to the grid, so that the voltage on the grid will vary sinoidally between zero and the anode supply voltage.

(4) The peak-to-peak a.c. voltage developed at the anode is transferred by a cathode follower to a phase-sensitive detector, the d.c. output voltage of which is related to the ionization current in the flame.

In Fig. 1, if C volts d.c. is supplied to the anode through a resistor of 2×10^9 ohms (corresponding to load-line BC), the peak-to-peak a.c. voltage developed at the anode is KC; similarly, if anode supply voltage E and load-line DE are chosen, the output voltage is LE.

If the ethylene flow-rate is doubled, the other flow-rates remaining unchanged, the height of the portion FJ of the curve doubles and the slope of AG doubles; consequently voltage LE is doubled but voltage KC only increases slightly. If anode supply voltage C and load-line BC are chosen, the detector will be approximately logarithmic for ethylene flow-rates greater than about 5×10^{-9} gm./sec. If anode supply voltage E and load-line DE are chosen, the detector will be linear up to, say, 10^{-7} gm./sec., and logarithmic for greater flow-rates. Thus, by suitable selection of anode supply voltage and load resistor the detector may be made approximately: (*a*) linear, (*b*) logarithmic, or (*c*) linear up to any desired flow-rate and logarithmic thereafter.

The flame ionization triode, like its vacuum counterpart, has both grid-anode capacitance and transit time effects. Because of the latter, considerable phase-shift occurs at frequencies above 20 c./s.; consequently it is preferable to apply sine waves rather than square waves to the grid. With the particular configuration used, the optimum frequency is 13 c./s. The grid-anode capacitance is of the order of 0·05 pF, which, at this frequency, gives a signal of about 1 volt peak-to-peak a.c. with no flame. This constant signal is removed by introducing an equal signal of opposite phase into the anode voltage supply. Constant background signals, caused by the hydrogen flame, are removed in the same way. This capacitance could be reduced by introducing further electrodes to form a tetrode or pentode; alternatively the grid may be earthed and the a.c. voltage applied to the jet.

One particular advantage of this type of detector is its high output. Applying 100 V. peak-to-peak to the grid and using a cathode follower with a measured input impedance of 2×10^9 ohms, followed by a phase-sensitive detector but without any intermediate amplifier, some 20 volts d.c. is available at the output of the phase-sensitive detector for an ethylene flow-rate of 10^{-8} gm./sec. The noise-level of the system in these circumstances is about 100 μV., which suggests an attainable detection limit of the order of 10^{-13} gm./sec.

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PHOTOLYSIS OF NITROMETHANE

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IT is reasonably certain[1] that the main primary step in the pyrolysis of nitromethane (at pressures below 200 mm.) is a breaking of the C—N bond and that a fraction of the methyl radicals produced form methane by hydrogen abstraction. Gray, Yoffe, and Roselaar[1] suggested that the mechanism of the photolysis is essentially similar, namely, a primary split:

$$CH_3NO_2 + h\nu \rightarrow CH_3\cdot + NO_2\cdot \qquad (1)$$

On the other hand, Brown and Pimentel[2] found that when nitromethane in solid argon at 20° K. was illuminated it isomerized to methyl nitrite, and considered that all the products resulted from the secondary photolysis of the nitrite. They stated "there seems to be no evidence preventing the assumption that the primary act of photolysis of gaseous nitromethane is the same as in the (argon) matrix", and as they found no evidence for the products nitrogen peroxide, methane or ethane they considered that (1) did not occur. The authors of both these papers claim that their primary step explains the photolysis products found for gaseous nitromethane by Hirschlaff and Norrish[3]. It is not obvious how either postulate explains the appearance of nitrogen as a major product. It seemed useful, therefore, to re-examine the gas phase photolysis and, in particular, to analyse the products for methane.

22 mm. of degassed nitromethane were photolysed at 27° C. in a quartz cell. (I am indebted to Mr. I. Brown of these Laboratories for a sample of pure nitromethane. Its mass spectrum was essentially identical with that quoted in "Mass Spectral Data of the American Petroleum Institute Research Project 44", Carnegie Institute of Technology, Pittsburgh, Pa.) The light source was a 250-W. medium pressure mercury arc used both directly and through a Jena *BG3* filter which cut out all light below 2500 Å. The products uncondensed by liquid air were expanded into 1-l. flask and analysed with a mass spectrometer. The results were recorded in Table 1 in units of 10^{12} molecules/ml. of illuminated volume/sec.

Table 1

Product	CO	N_2	NO	H_2	CH_4	C_2H_6
Light			1·7			
None		0·9				
		0·1	0·5			
Full arc	159	23	76	45	15·0	0·6
" "	144	18	85	46	14·5	1·1
" "	161	21	79	45	14·2	0·5
Filter	32	4·6	14	9·3	0·81	0·04
"	13	4·2	20	5·3	0·86	0·07
"	23	3·3	19	6·8	0·80	0·09

Although the amount of methane was small the analysis was quite unequivocal since it depended on peaks at masses 15 and 16 to which there was practically no contribution from other gases present. The analysis for nitrogen was less accurate since it depended on the residual at mass 14 after subtraction of the contribution from nitric oxide and methane. Nevertheless its identification was quite certain. Small peaks, about twice the height of the mass spectrometer background, at masses 26 and 27 were in the right ratio to be produced by ethane and were tentatively so assigned.

A second fraction, of products uncondensed at −79° C., was examined, but the mixture was too complex for quantitative analysis. Nitrogen peroxide, carbon dioxide, nitric oxide and methyl alcohol were identified positively. A peak at mass 29 together with other residuals seemed fairly certainly due to formaldehyde. After subtraction of the contribution from carbon dioxide, residuals at masses 44 and 45 were in the ratio of 25 : 1 which agreed exactly with the ratio of these peaks in a sample of formaldoxime. It appeared unlikely that any of the mass 44 peak was due to nitrous oxide.

A solid white polymer was also formed during the photolysis; wildly erratic results for the carbon monoxide and hydrogen analyses were obtained until the polymer was removed after each run. The product obtained by heating this polymer gave major peaks at masses 45, 30, 29 and 15 consistent with the view that it was a mixture of formaldehyde and nitrosomethane.

Except for the nitrogen, all the products found are those to be expected from the primary split (1). Methane is formed by:

$$CH_3\cdot + CH_3NO_2 \rightarrow CH_4 + \cdot CH_2NO_2 \qquad (2)$$

and the $\cdot CH_2NO_2$ radical probably splits to give formaldehyde and nitric oxide:

$$\cdot CH_2NO_2\ (? + M) \rightarrow CH_2O + NO. \qquad (3)$$

Oxygen is present from the secondary photolysis of nitrogen peroxide, and it is well known[4] that oxidation of methyl radicals leads to carbon monoxide, carbon dioxide, methyl alcohol and formaldehyde. The reactions:

$$CH_3\cdot + NO_2 \rightarrow CH_3O\cdot + NO \qquad (4)$$

$$CH_3\cdot + CH_3NO_2 \rightarrow CH_3O\cdot + CH_2NO \qquad (5)$$

probably occur also. The hydrogen is produced by the secondary photolysis of formaldehyde. Finally, Coe and Doumani[5] have shown that the reaction of methyl radicals with nitric oxide leads to nitrosomethane, formaldoxime and their solid polymers. Thus the evidence obtained from these analyses gives convincing support to the view that (1) is the major primary step in the gas-phase photolysis. This postulate is consistent with the results of Brown and Pimentel[2] if it is assumed that in the gas phase methyl radicals and nitrogen peroxide disproportionate by (4) but that in the 'cage' of the solid matrix at 20° K. they combine to form methyl nitrite rather than the original nitromethane.

This work gives no support to the conclusion of Hirschlaff and Norrish that nitrogen is the major product of the photolysis. It is not clear whether their 'nitrogen' was analysed for methane and hydrogen. Nitrogen is, however, a minor product, as it is also in the photolysis of tetranitromethane[6]. Except that energy considerations would suggest a heterogeneous reaction, the mechanism for nitrogen production in these photolyses is obscure.

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BIVALENT METAL IONS AS THE COUPLING FACTOR BETWEEN CELL METABOLISM AND THE RATE OF CELL MUTATION

By Dr. G. G. JAYSON

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ONE of the most important interactions which take place in the cell is transphosphorylation. The way phosphates enter into the energy cycles and their presence in nucleic acids has been investigated by a great number of workers. What has become clearer recently is the importance of the divalent metal ions in this process.

Thus, Ashwell and Hickman[1] showed that calcium accelerates the breakdown of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) in spleen homogenates. X-rays were found to have a similar effect. As the radiations are known to deactivate enzymes, their action was ascribed to the destruction of cellular material allowing greater contact between the enzymes and ATP. More recently, Lowenstein[2,3], has demonstrated that enzymes are not necessary for the hydrolysis of ATP to ADP. Divalent ions, for example, Ca^{2+} and Mg^{2+}, alone were sufficient to bring about the transformation:

$$\text{ATP} + \text{orthophosphate (P 32)} \xrightarrow{Ca^{2+} \text{ and } Mg^{2+}} \text{ADP} + \text{pyrophosphate}$$

where: orthophosphate $= 5 \times 10^{-3}$ M; ATP $= 1 \times 10^{-2}$ M; $Mg^{2+} = 1 \times 10^{-2}$ M.

The formation of pyrophosphate at these concentrations in aqueous solution by means of a non-enzymic reaction is quite remarkable. Lowenstein explained the catalytic action of the bivalent metal ions by the complex they can form with ATP; although he is not sure whether Ca-ATP, Ca-ATP-Ca or Ca-ATP-Mg is the important entity leading to ADP formation.

The hydrolysis of condensed phosphates such as pyro- and tri-polyphosphate has been investigated in some detail[4]. It is markedly affected by changes in *p*H and the presence of metal ions (particularly calcium). Van Wazer[4], describing work by Griffith at Monsanto, has explained this on the basis of an 'activated complex' which takes the form of the metal chelate. On that basis, Ca-ATP ought to be the complex which leads to ADP.

The behaviour of the condensed phosphates and their organic derivatives (for example ATP, ADP) with respect to calcium ions in aqueous solution appears to be similar[5]. This is not surprising because the dominant ionic feature is the polyphosphate chain. To this must be added what is now known of the interactions between condensed phosphates, 'hardness' in water and metals on surfaces[6]. Fig. 1 shows the effect of mixing solutions of condensed phosphates and calcium. It is possible to get an insoluble salt $Ca_2P_2O_7$, or a soluble complex $CaP_2O_7^{2-}$.

$$(1)\ \xrightarrow[\text{in phase diagram}]{P_2O_7 \text{ into calcium}} (a)\ P_2O_7^{4-} + 2\,Ca^{2+} \longrightarrow \underset{\text{insoluble salt}}{Ca_2P_2O_7}$$

$$(b)\ P_2O_7^{4-} + Ca_2P_2O_7 \longrightarrow \underset{\text{soluble complex}}{2CaP_2O_7^{2-}} \xrightarrow{\text{hydrolysis}}$$

$$(2)\ \xrightarrow[\text{in phase diagram}]{\text{Calcium into } P_2O_7} (a)\ Ca^{2+} + P_2O_7^{4-} \longrightarrow \underset{\text{soluble complex}}{CaP_2O_7^{2-}} \xrightarrow{\text{hydrolysis}}$$

$$(b)\ Ca^{2+} + CaP_2O_7^{2-} \longrightarrow \underset{\text{insoluble salt}}{Ca_2P_2O_7}$$

Tripolyphosphate behaves in a similar manner, but does not show as great an insoluble phase. Whether the complex is hydrolysed or not will depend on the *p*H of the solution. Once the insoluble salt has been formed, it is difficult to bring it back into solution again. In fact, it has been found that five times the stoichiometric quantity of polyphosphate is needed[7]. It follows that, whenever the calcium phosphate system passes through an insoluble phase, a time-lag must occur before the calcium can be brought back into solution and catalytic service. Also, once the insoluble calcium salt has formed, we are really working with an inhomogeneous system, as observed in detergency[6]. If calcium were to be precipitated in the cell as another insoluble salt (for example, calcium fatty acid) the same problems of solubilization would arise. The exact position taken up by the phosphates, ATP and ADP, during transphosphorylation and the build-up of nucleic acids must therefore be determined by the positions of the divalent metal ions and local conditions of *p*H.

These results, then, explain why small changes in calcium and ADP concentration have such a marked effect on the control of respiration in the cell[8]. Furthermore, the control points in phosphorylation would in fact be positions where divalent metals are to be found[9]. It can be seen from this that the part which calcium and magnesium ions play in cell metabolism is of the utmost importance.

In addition to the above it has been found that in the nucleus calcium and magnesium ion hold together sections of chromosomes[10]. That is, changes in the concentration of the ions will determine the degree of chromosomal aberration. Ethylenediamine tetraacetic acid (EDTA) was found to produce breaks in the chromosomes of *Vicia faba*[11]. *Tradescantia* grown in calcium-deficient diets showed similar results[12]. If the effect of calcium and magnesium ion concentration on chromosomal aberration were to be added to that of its action on the phosphorylating metabolism, the pattern depicted in Table 1 would emerge.

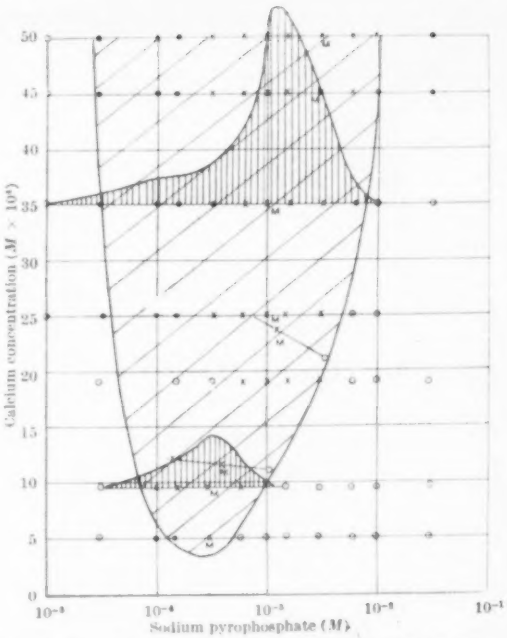

Fig. 1. Phase diagram of calcium pyrophosphate at 23° C. ×, Precipitate; ○, clear solutions; ●, doubtful; ///, intensity of phase; *M*, maximum in any series

Fluctuations in the concentrations of divalent metal ions in the various parts of the cell could arise through precipitation of an insoluble salt (for example, CaADPCa) rather than formation of the soluble complex (as in the chemistry of Ca^{2+} and $P_2O_7^{4-}$; see above[6]). Calcium ions removed by the former method would be virtually lost to the aqueous phase because of the time-factor involved in bringing them back into solution. This would also hold for calcium ions precipitated on walls of cell, mitochondria and nucleus. In effect, the system would have lost a homogeneous catalyst and possibly gained a heterogeneous one.

Table 1. Effect of Fluctuation of Calcium Concentration on the Metabolism and Mutation-Rate of the Cell

Ca^{2+} concentration in the cell	Metabolic-rate	Rate of mutation	Mitosis rate of reproduction	State of cell
± Ca^{2+}	Normal rate of ATP breakdown	Normal number of chromosomal aberrations	Normal rate of mitosis	Normal integrated cell
− Ca^{2+}	Decrease in ATP breakdown	Increase in chromosome* aberrations (including mutant which allows cell to operate at lower Ca^{2+} concentration)	Mitosis retarded	Differentiation of the integrated cell (benign)
+ Ca^{2+}	Increase in ATP* breakdown (further increase for mutant which allows all to operate at lower Ca^{2+} concentration)	Decrease in chromosome aberrations (that is, holding on to any mutant produced in − Ca^{2+} phase)	Mitosis increased (particularly for mutant)	Rapid (malignant) growth of non-integrated cell

* Frequency of sensitive stage in cell division increased, that is, particularly sensitive to X-rays

—— show the effect of cycle in Ca^{2+} concentration.
* shows points where X-rays have an effect. They will also increase the permeability of the membranes.

If the cell, in order to carry on with its metabolism (transphosphorylation) removes calcium or magnesium out of the nucleus into the cytoplasm, it will increase the number of chromosomal aberrations. One of these may be a mutation which allows the cell to live with a metabolism at the new, lower calcium-ion concentration. When the original calcium-ion concentration is restored (by complexed calcium entering from outside the cell, or the calcium precipitate eventually redissolving into the aqueous phase) the cells with the 'low calcium metabolic' mutant would show a much higher metabolism and rate of mitosis. As increased calcium-ion concentration reduces chromosomal aberration, there would be no possibility of reversing the mutation or adapting it as before. This sequence of events is shown in Table 1.

Neoplastic growth induced by other agents would exert its effect at some point in the calcium control. For example, some carcinogens may induce this calcium cycle by interfering with the respiratory (electron and proton transfer) system, for example, phenanthrene. On the other hand, such compounds may cause precipitation of the calcium ions on the cell walls. X-rays will produce interactions at a number of points in the cell and these are shown (by an asterisk) in Table 1.

It should be possible to encourage the formation of 'rapidly metabolizing' mutants by submitting the cells to a diet which is alternatively low and high in calcium content. Conversely, mutation of normal cells should be suppressed by the introduction of a calcium 'buffer'[12] (for example, complex of calcium) into the cell. The way to decelerate the high rate of mitosis of mutant cells would be withdrawal of Ca^{2+} (for example, by means of EDTA). 'Synkavit' (tetrasodium 2-methyl-1, 4-methyl-1, 4-naphthohydroquinone diphosphate) which has been used by Mitchell[11] for the treatment of skin cancer and rectal adenocarcinoma probably acts in the same way. However, application of Ca^{2+} or Mg^{2+} depressants would induce chromosomal aberrations in normal neighbouring cells and, therefore, would have to be carried out locally. (From a medical point of view, the agent for removing calcium would have to be injected into the tumour or, if possible, the tumour would have to be bathed with such a solution.) There are obviously a great number of biological and medical experiments which could be based on the theory expressed above.

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REVERSAL OF DIURNAL TEMPERATURE RHYTHMS IN MAN

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RECENTLY, while working with the University of Nottingham Department of Pharmacy Expedition to Spitsbergen, the opportunity arose to study the diurnal temperature variations in a group of 6 men and 2 women, who were subjected to sudden reversal of their activity-sleep and light-darkness schedule. The subjects lived a controlled life of activity, diet, time of meals and they went to bed and awoke at set times each day. Spitsbergen provides an ideal environment for this type of work during the summer months because of the 24-hr. daylight and even temperatures. It was necessary for the subjects to sleep with blindfolds on to ensure the alternation of light and dark. They retired at 22.30 hr. each night and arose at 07.00 hr. under control conditions. On any test day, body temperatures were recorded every 3 hr. from rising until 22.00 hr. and again on rising the following morning.

Temperatures were taken using clinical thermometers placed in the armpit. It would have been desirable, but was impossible in the circumstances, to record rectal temperatures. Oral temperatures were found to be unreliable in an environment where facial cooling was appreciable so that skin temperature was the only possibility, and the armpit a very convenient site. After 4 test days on the control routine (periods 1–4) the subjects reversed their routine exactly on a 12 hr. basis. Thus they arose at 19.00 hr., worked through the night and retired at 10.30 hr. By sleeping with blindfolds on, the day became an artificial 'night' and the night became 'day'. Due to the 24 hr. daylight, there was little difference between night and day. In order to minimize the stress during reversal the subjects slept all night on the last control period, had breakfast, and then retired to sleep again, from 10.30 to 19.00 hr. From this point they arose, had breakfast again and worked through the night. Temperature records were made as before on test days, 2, 4, 6 and 8 days after reversal (periods 5–8). After 14 days on this routine, a reversal back to normal was made and the adaptation of temperature rhythms noted. Tests were performed again on four alternate days after the change (periods 9–12).

Daily routine

Periods 1–4 and 9–12 (hr.)	Periods 5–8 (hr.)	
06.50	18.50	Reveille, blindfolds lifted.
07.00	19.00	Body temperature taken, weather records made.
07.30	19.30	Breakfast.
10.00	22.00	Body temperature taken.
13.00	01.00	Body temperature taken. Lunch.
16.00	04.00	Body temperature taken. Tea.
19.00	07.00	Body temperature taken, weather records made. Dinner.
22.00	10.00	Body temperature taken.
22.30	10.30	Subjects to bed, blindfolds on.
07.00	19.00	Body temperature taken.

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Table 1. Diurnal Body Temperature Rhythms expressed in Degrees Fahrenheit and standard Deviation

Time-Period	07.00	10.00	13.00	16.00	19.00	22.00	07.00
1–4	96·6 ± 0·9	97·3 ± 0·9	97·0 ± 0·9	97·3 ± 0·7	97·2 ± 0·8	97·4 ± 0·8	96·7 ± 1·0
	19.00	22.00	01.00	04.00	07.00	10.00	19.00
5	97·1 ± 0·9	97·1 ± 0·8	97·2 ± 0·6	96·9 ± 0·9	97·0 ± 0·9	96·8 ± 1·0	96·9 ± 0·9
6	96·5 ± 1·0	97·3 ± 0·9	96·9 ± 0·8	97·2 ± 1·0	97·1 ± 1·0	97·1 ± 1·0	96·9 ± 0·9
7	96·6 ± 0·9	97·8 ± 0·7	97·4 ± 0·8	97·4 ± 0·8	97·3 ± 0·9	97·7 ± 0·9	96·7 ± 0·9
8	97·3 ± 0·7	98·0 ± 1·0	97·4 ± 1·0	97·7 ± 0·9	97·2 ± 0·8	97·5 ± 0·6	97·1 ± 0·8
	07.00	10.00	13.00	16.00	19.00	22.00	07.00
9	97·2 ± 0·6	97·5 ± 0·8	96·9 ± 1·1	97·2 ± 1·1	97·0 ± 1·0	97·1 ± 1·0	96·8 ± 0·9
10	97·1 ± 0·7	97·9 ± 1·1	97·7 ± 0·9	97·7 ± 0·5	97·1 ± 0·9	97·6 ± 1·0	96·7 ± 1·0
11	96·9 ± 0·7	97·5 ± 1·1	97·5 ± 0·9	97·6 ± 0·9	97·5 ± 0·7	97·4 ± 0·7	97·0 ± 0·5
12	97·0 ± 0·9	97·9 ± 0·9	98·0 ± 0·8	97·8 ± 0·9	97·6 ± 0·7	97·6 ± 0·7	97·3 ± 0·5

The results are shown in Table 1, where the average temperature during each period is recorded.

The diurnal variation exhibited by subjects, under these particular experimental conditions, was characterized by a rapid rise of temperature from waking to 10.00 hr. followed by a decrease, though temperatures remained high for the rest of the day. A slight rise of temperature occurred after each meal though one may not assume any significance in this correlation. During the night, temperatures fell to low values. In period 5, two days after the reversal of routine, the waking temperature was high and no rise occurred in the following three hours. A slight rise to lunch-time was followed by afternoon and evening temperatures which were below the waking value. It was clear that the temperature rhythm had not become adapted, by reversal, to the new time-scale, and that the temperatures recorded from lunch-time on were 'night values'. During the second 'night', however, the temperature rose slightly and to the normal waking value.

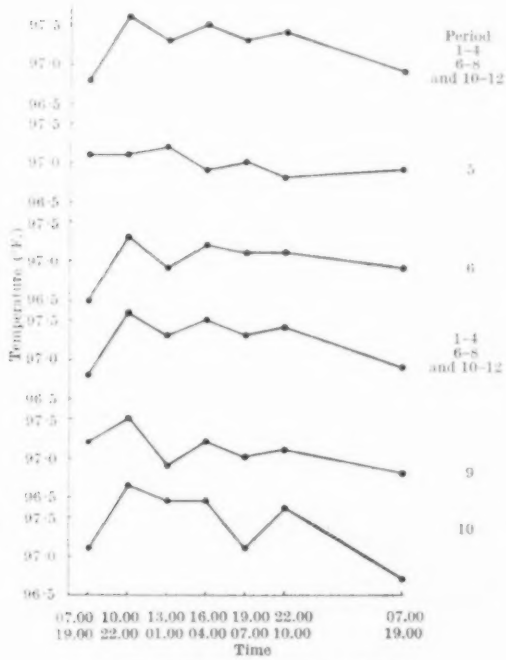

Fig. 1. Diurnal temperature variation

In period 6 a fairly typical diurnal variation was observed with a sharp rise after waking, a slight fall following, raised 'daytime' values and a fall at 'night'. Thus reversal of the diurnal temperature rhythm had occurred within 3–4 days of reversal of routine. After adaptation and two further test days a return to normal was accomplished by a further 12-hr. time shift. Period 9 represents the test day 2 days after the reversion to normal. The waking temperature was elevated, as in period 5, and only a slight rise occurred during the next 3 hr. The pattern from there onwards was similar to the control pattern except for lower temperatures and only a slight night fall. In period 10 a steep waking rise and night fall were both present. Reversion of rhythm to normal had again occupied 3–4 days. The pattern of diurnal temperature variations are shown in Fig. 1.

A diurnal variation of body-temperature was noted shortly after the introduction of thermometers into clinical practice. Warter[1], for example, recorded minimum temperatures in the early morning and maxima in the evening. Ogle[2] in the same year recorded afternoon maxima and also stated that the variations were greater in men than in women. A review of the earlier literature has been given by Woodhead and Varrier-Jones[3]. They pointed out the pitfalls of this type of work and emphasized the importance of sleep and light, the conditions and means of temperature measurement, and the fact that even when subjects are in bed for 24 hr. or more the diurnal temperature rhythm still persists.

Several investigations into the diurnal rhythm, and particularly its persistence, have been made. Kleitman and Doktorsky[4] have shown that the sleep-wakefulness cycle is an individually acquired phenomenon depending on the cerebral cortex for establishment and maintenance, and that the body temperature rhythm parallels the development of the sleep-wakefulness cycle. Observations on infants confirm the relationship between activity and temperature rhythms, though Metz and Andlauer[5] were unable to correlate temperature curves with variations of energy metabolism. That the temperature rhythms are developed by a process of acculturation is undoubted. Experiments on rabbits have

shown that feeding habits play an important controlling part[6]. In man, fasting has been shown not to eliminate the rhythm. Kleitman found that diurnal temperature curves may differ in individual subjects with extremes of morning or early evening peaks[7], and also that after waking the morning rise in temperature may be followed by a 'plateau' effect for the remainder of the day and early evening[8]. In monkeys, the temperature rhythm has been reversed by reversal of lighting, while gradual extinction occurred after one week in darkness[9]. The diurnal rhythm has been reversed in man by reversal of daily routine[7], though resistance to change has been noted. Individuals may vary greatly in their adaptability, some failing to adapt after three weeks on night work, and others become adapted in a few days. Burton[10] found that his own temperature rhythm required 3–4 days to become accommodated with English time after a flight from Canada. This involves a time-shift of 5 hours in the daily routine.

In Fig. 1 selected periods are used to illustrate the diurnal variation, the intensity of the morning rise and the adaptation to a reversed routine. Reversal of the diurnal temperature curve occurred in 3–4 days and all the subjects showed the same general pattern. The normal temperature observed on waking on the third morning after reversal of routine is indicative that the rhythms were becoming stabilized on the new routine while the typical post-waking rise was present four days after reversal. Throughout the test periods no variations occurred in environmental temperatures sufficient to invalidate any comparison between periods. The temperature variations are clearly not directly dependent on physical activity or food intake because reversal did not occur immediately when the activity-sleep routine was reversed. They depend ultimately on habit and environment, with light as the most likely controlling stimulus. The time of adaptation reported here agrees well with Burton's observation of 3–4 days to become adjusted to a 5-hr. time-shift.

One further point of interest is that etiocholanolone causes a rise of body temperature after injection which may last for several hours[11]. In view of the diurnal variation of ketosteroid output which is greater by day than by night it was thought that this might be influencing the temperature rhythm. In 4 of the male subjects of this work, a simultaneous study of keto- and ketogenic-steroid excretory patterns was made. Reversal of the keto- and ketogenic-steroid pattern occurred in 2 and 6–8 days respectively. Because the temperature rhythm was reversed in 3–4 days it seems unlikely that these steroids play an important part in the control of body-temperature rhythms though this does not exclude the possibility that they exert some effect upon temperature-levels.

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TWO LINKED GENES SHOWING A SIMILAR TIMING OF EXPRESSION IN MICE

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TWO single gene mutations in the mouse show similar timing of expression during post-natal development. The two mutant alleles were found to produce nearly normal phenotypes until some critical time after birth following which normal differentiation is arrested. The first of these genes, originally reported by Morrow, Greenspan and Carrol[1] in *C3H* mice is expressed in the recessive state by a deficient production of the enzyme β-glucuronidase[2,3]. It has recently been demonstrated that homozygous mutant animals (*gg*) produce the enzyme in some tissues, notably liver, only until a certain post-natal stage, after which a progressive decline sets in and the enzyme ceases to accumulate further. Wild-type (*GG*) stocks produce the enzyme throughout growth[4]. A representative experiment is shown in Fig. 1.

The second mutation is that responsible for a recessive hereditary visual cell degeneration recently described from several laboratories[5–9]. At birth, the retinæ of mice homozygous for the recessive allele (*rd*) do not differ significantly from those of control animals, and post-natal differentiation at first proceeds normally. Beginning, however, at the onset of the most rapid phase of visual cell differentiation on the tenth day, and just after the visual cells have become minimally responsive to light stimulation, the cells die. By the fourteenth day, 50 per cent of the visual cells have disintegrated; at three weeks of age practically none remain, and the animals are blind. This process has been described in detail[8].

Both mutations are characteristic of *C3H/Ha* mice, and the experiments to be described show that in addition to sharing a similar pattern of expression, they are closely linked on the same chromosome. They do not, however, appear to control the same physiological process.

In these experiments β-glucuronidase was assayed by a modification of the phenolphthalein glucuronide procedure of Talalay *et al.*[3]. Activity is expressed as μgm. of phenolphthalein formed per hour when assayed at 56° C. Presence or absence of visual cell degeneration was ascertained by the electrical response to light stimulation (electroretinogram) and histological examination[8].

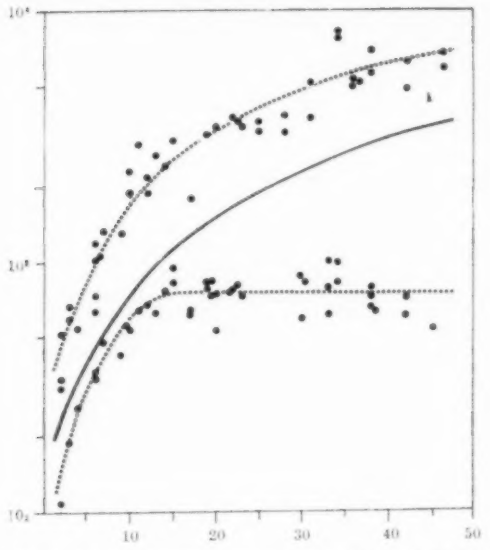

Fig. 1. The total enzyme content of liver plotted as a function of days of age in a population of mice segregating for the *g-G* alleles (that is, the progeny of a *gg* × *Gg* cross). Each point represents a single mouse and enzyme activity is expressed as total units per liver. The solid line is the growth curve of liver in this population expressed as mgm. × 10 dry weight. The dashed lines are drawn to indicate the growth curves of the enzyme in the two classes of progeny

It is apparent from Table 1, which lists the results obtained with a number of mouse strains, that *C3H/Ha* mice show both mutations. In *AKR*, *C3H/St* and *CFW* lines the *g-G* and *rd-Rd* allelic pairs are expressed independently of each other. The *C3H/St* and *CFW* are blind, but have high liver glucuronidase, whereas the *AKR* animals show no visual cell degeneration, but have low glucuronidase levels. Evidence that the two loci do not affect a common function was also obtained from examination of *Ha/ICR* Swiss mice. The *Ha/ICR* Swiss are a deliberately non-inbred population. Among mice of this stock approximately 13 per cent (59/444) were

Table 1. GLUCURONIDASE CONTENT AND SIGHTEDNESS OF MOUSE STRAINS

Strain	No. of animals	Liver glucuronidase (units/mgm. wet wt.)	Sightedness
Lat	3	5·4	+
IPBR	3	6·7	+
I	3	6·7	+
5-PRUNT	3	6·8	+
H	3	7·0	+
CBA/St	6	7·4	+
CFW	3	7·6	0
C/St	3	7·8	+
*B1**	3	7·9	+
CHI	3	8·0	+
C3H/St	9	8·3	0
JK	3	8·8	+
PBR	6	9·2	+
A	3	9·2	+
BrS	3	9·4	+
PIN	3	9·5	+
N	3	9·9	+
F	3	10·3	+
C57	3	10·3	+
2-PRUNT	3	10·5	+
DBA/2	10	11·6	+
LOW	3	12·2	+
AK	6	0·9	+
C3H/Ha	10	1·6	0

Table 2. LINKAGE OF GLUCURONIDASE AND HEREDITARY VISUAL CELL DEGENERATION

gG rd Rd × *gg rd rd*

	Blind	Sighted
High enzyme	1	33
Low enzyme	23	2

The crosses used were (*C3H/Ha* × *DBA/2*) × *C3H/Ha* and (*DBA/2* × *C3H/Ha*) × *C3H/Ha*. In crosses of the first type, the *C3H* carried the milk factor; in those of the second type, they did not.

blind and all (47/47) had high enzyme. That the genetic factor responsible for the visual defect in Swiss mice involves the same locus as that in the *C3H* is indicated by the failure (0/102) to observe sighted offspring of *C3H/Ha* × Swiss matings. Also, the behaviour of the *rd* allele in various other crosses was independent of its origin.

The F_1 animals of the cross *C3H/Ha* × *DBA/2* are heterozygous at both loci and were indistinguishable from the dominant *DBA/2* parent. Examination of the offspring of a backcross of the F_1 to the *C3H* parent showed that the two loci are linked, being approximately 5 cross-over units apart (Table 2). Other linkages of the *Rd* or *G* genes are not known. In the present experiments neither factor was linked to sex, or to the coat colour genes *A*, *B* and *D*. In addition, these loci were not linked to the H-2 genic complex[10]. It has been reported that *Rd* is not linked to pigmy[11] or silver[12], and *G* is not linked to the colour genes, *A*, *B* and *C*[13]. These facts eliminate their location on linkage groups I, II, IV, V, VIII, IX or XX[13].

For the *rd* allele, although differentiation of the visual cells is blocked and cell death ensues, no effect is observed in the other retinal cells of the same ontogenetic origin, the pigment epithelium, the bipolar cells, and the ganglion cells. Similarly, for the *g* allele, although enzyme ceases to appear in the liver after the seventeenth day, enzyme production in the spleen is maintained throughout its growth. For both of these loci expression of the recessive alleles involves a nearly normal function until in some tissues, at a time characteristic for each, functional failure occurs. Despite these similarities, the two genes are separable by crossing-over and do not appear to involve a common function.

Although the genetic linkage observed may be purely fortuitous, in view of the similarities of the two loci the possibility must be considered that their linkage reflects the functional organization of the chromosomes.

A number of reports, stemming from the original observation of Beerman[14], have demonstrated that characteristic chromosomal regions swell and then shrink in the cells of particular tissues at certain stages in development, and the experiments of Briggs and King[15] suggest that differentiation may involve a concomitant loss of genetic totipotency among tissue nuclei. In the light of these findings several investigators have again proposed that the primary control mechanism in differentiation is a nuclear process involving differential gene activation or genetic segregation among the developing cells. Strong genetic support for a nuclear control comes from the experiments of McClintock[16–18], showing that a class of genetic elements exists that control the time during development at which secondary genetic events may occur.

The report of Callan and Lloyd[19] on the heterozygosity of puffing suggests that different alleles of the same gene can react differently to such control mechanisms during development, as appears to be

the case with glucuronidase and hereditary visual cell degeneration. They found that in the oocytes of *Triturus* neither, one, or both members of a chromosome pair may show puffs at a given locus. The finding that although cells from different individuals differed widely, all oocytes from the same animal showed identical configurations at all sites, indicated that pairs of alleles were involved in which one member forms puffs and the other does not.

If the findings reported here are considered in relation to these cytogenetic phenomena, they suggest that particular chromosomal regions may be specialized to control the timing of expression of the loci within them, and thus provide a nuclear regulation of differentiation. More serious consideration of this hypothesis must await the finding of other cases of close genetic linkage between genes showing similar patterns of physiological expression.

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AMINOGLYCOLIPIDS AND GLYCOPROTEINS OF HUMAN BRAIN

I. Quantitative Fractionation of Brain Glycolipids by Silicic Acid Chromatography

WHILE there are a number of methods by which the glycolipids of mammalian brain may be individually isolated and purified, a method would be desirable whereby all the glycolipids of brain could be quantitatively separated and recovered, and their concentrations and other properties compared between individual brains. This is of interest not only with reference to the pathological lipodystrophies[1], but also with reference to the possibilities for chemical individuality of these substances in normal brain[2].

Since silicic acid chromatography has been found to be useful in the separation of both synthetic and native lipid mixtures[3], it was applied to the present problem. Whereas gradient elution of selected lipids from silicic acid columns had been found effective in the quantitative separation of a selected group of brain lipids[4], there was no evidence to indicate that a total hot alcoholic extract of brain lipids might be resolved quantitatively by these methods. With the desire to obtain quantitatively reproducible fractions which might then be compared in brain specimens from different individuals, a stepwise elution procedure was adopted, with quantitative exhaustion at each step of all the solids which could be removed from the column with a given solvent mixture.

Fresh whole bovine cerebral hemispheres, freed of investing meninges and blood vessels, were extracted with hot methanol as previously described for the isolation of brain ganglioside[5]. This procedure has been used by numerous investigators[6] for the preliminary extraction of cerebrosides and related glycolipids. In the course of preparation of brain ganglioside the filtered hot methanolic extract is allowed to stand at 4° C. for 48 hr., during which time a precipitate forms. The precipitate is filtered in the cold, then taken up at room temperature with chloroform – methanol (2 : 1), and partitioned twice with water. The upper phase contains the bulk of the brain ganglioside and other hexosamine-rich material present in the precipitate (and discussed in the next communication), and the resultant lower phase contains the other glycolipids and phospholipids. This lower phase material is taken to dryness by vacuum distillation at 60° C., taken up with minimal amounts of chloroform – methanol (2 : 1), allowed to dry at room temperature, and stored in a vacuum desiccator.

Silicic acid (Baker's reagent grade) was dried overnight at 105° C. To columns 40 mm. × 600 mm. with a fritted disk of coarse porosity was added a thoroughly mixed suspension of 250 gm. of silicic acid and 500 c.c. of chloroform – methanol (2 : 1) and additional chloroform – methanol added until a total of 1,250 c.c. were used. When the chloroform – methanol had run through to the level of the silicic acid, 1,000 c.c. of chloroform was added until the column was translucent and free of opacities[4]. The column was charged with 1,000 or 2,000 mgm. of lower-phase material in chloroform. The solvents utilized for elution were first chloroform, then mixtures of chloroform – methanol with increasing amounts of methanol, and finally pure methanol. The entire fractionation was done in a constant-temperature room at 25 ± 2° C. Fourteen fractions were thereby obtained (Fig. 1). In addition to the good general separation previously observed with more limited mixtures between glycolipids and phospholipids[4], excellent quantitative recoveries in terms of dry weight, hexose[7], phosphorus[8], and nitrogen[9] were achieved (Table 1). This quantitative result provides a possibility for a more reliable comparison of absolute amounts of these separate constituents in both human and animal brain preparations.

Fig. 1 shows that fractions III–VI are completely phosphorus-free, yet high in content of hexose. That these are distinct fractions is demonstrated: (1) by the fact that each fraction is exhaustively removed from the column by its particular solvent mixture

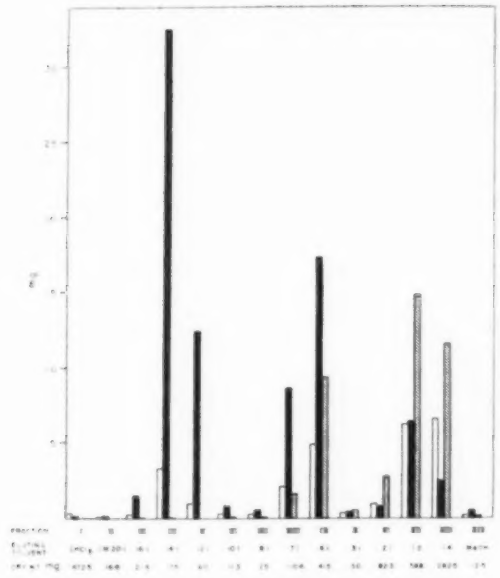

Fig. 1. Stepwise quantitative elution of brain glycolipids. Nitrogen, □; hexose, ■; phosphorus, shaded

until no further dry weight is obtained; (2) the nitrogen percentage compositions as well as those of hexose differ appreciably in each of these fractions; (3) these fractions can be distinguished by further partition in terms of their solubility in chloroform – methanol (2 : 1) at 4° C. Thus, for example, fractions IV and V under these conditions both yield precipitates with concentrations of nitrogen (1·73 per cent) and hexose (21·3 per cent) suggesting a cerebroside; but the remaining supernatants differ markedly in fractions IV and V; thus the material soluble in cold chloroform – methanol in fraction IV contains 1·8 per cent nitrogen, 22·4 per cent hexose, whereas the material soluble in fraction V contains 1·5 per cent nitrogen, 14·3 per cent hexose. Similar observations apply to each of the other fractions shown in Fig. 1 which contain hexose. Therefore, with respect to elution behaviour, total percentage composition, and composition of sub-fractions, the fourteen major fractions collected by the stepwise procedure described are distinctive.

When all the fourteen fractions obtained were examined for their content of neuraminic acid, this substance was found in only one of the fractions, fraction XII. That this represents brain ganglioside which has not (quantitatively) passed into the upper phase during the preliminary separation is suggested by the fact that purified brain ganglioside[5] was found to be eluted only at this stage when tested by itself on the identical silicic acid column. Thus, at chloroform – methanol ratios of 1 : 1, 1 : 1·5 and 1 : 2, respectively, brain ganglioside is obtained quantitatively. It is of interest to note that each of these three sub-fractions obtained from purified brain ganglioside shows varying concentrations of neuraminic acid and hexosamine. This variation would be in accord with the previous observation in this Laboratory that our preparation of brain ganglioside contains more than one immunochemically distinct substance[7], despite the fact that it demonstrates electrophoretic and ultracentrifugal homogeneity and homogeneity in several solvent systems. It must be considered, however, that despite the apparently non-traumatic nature of the silicic acid chromatography employed, partial degradation of a unitary brain ganglioside material might have occurred. In the case of more harsh solvent procedures utilized by others, in one case[8] acidic solvents known to promote degradation were utilized, and in another case[9] no fraction or sub-fraction of 'brain ganglioside' was obtained with concentration of carbohydrates equal to that of the purified brain ganglioside prepared in this laboratory. While there is no evidence that silicic acid chromatography as used here results in actual degradation in terms of neuraminic acid and hexosamine (recovery 104 and 105 per cent, respectively), the possibility that intramacromolecular disjunction occurred in such a way as to produce fragments with varying relative concentrations of neuraminic acid and hexosamine cannot as yet be ruled out.

Table 1. Quantitative Recovery of Dry Weight, Nitrogen, Hexose and Phosphorus in Stepwise Elution of Brain Glycolipids from Silicic Acid Columns

Constituent	Type of brain	Amount added to column (mgm.)	Amount recovered (mgm.)	Percentage recovery
Dry weight	Bovine	2000	2088·9	104·4
	Bovine	2000	2113·6	105·7
	Human	2000	2239·8	112·0
Nitrogen	Bovine	26·4	29·3	111·1
	Bovine	26·4	26·7	101·0
	Human	27·3	30·2	110·7
Hexose	Bovine	81·8	82·2	100·4
	Bovine	81·8	84·7	103·5
	Human	81·5	80·2	98·4
Phosphorus	Bovine	42·7	41·1	96·2
	Bovine	42·7	40·9	95·9
	Human	51·8	50·5	97·4

The presence of amino-acids has been tested for, both in the unhydrolysed and in the hydrolysed fractions obtained from the stepwise chromatography of lower-phase material. The combined precipitates obtained from fractions IV and V show the presence of amino-acids before hydrolysis to the extent of 0·17 per cent; this is increased by hydrolysis with 6 *N* hydrochloric acid for 36 hr. to 0·94 per cent. The filtrates show the presence of 0·13 and 0·80 per cent in the unhydrolysed fractions IV and V, and 1·5 and 1·74 per cent in the hydrolysed samples respectively. The presence of bound amino-acids in at least some of these glycolipid fractions of brain must therefore be considered as in the case of the *Drosophila* lipids[10], and is being further investigated.

The stepwise silicic acid chromatography method described therefore provides a quantitative means of separating, accounting for, and comparing individual glycolipid and phospholipid fractions from mammalian brain. The application of these quantitative methods to human brain specimens has been accomplished with the same yields (Table 1) and will be reported in detail elsewhere. These methods thus provide an opportunity of comparing quantitatively the presence of individual glycolipid fractions in individual brain specimens.

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Institute of Mental Health, United States Public Health Service (L. A. F.).

SAMUEL BOGOCH
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2. Hot Methanol Extraction of Human Brain Aminoglycolipids

THE term 'aminoglycolipids' is suggested here to include the family of substances the basic constituents of which are glycolipid in nature, and the additional constituents of which include some amino-carrying components such as a hexosamine or neuraminic acid and its derivatives, or amino-acids, or any combination of these three classes of substances. This group of substances would therefore include cerebroside-like substances with extra hexose units per mole of sphingosine[1,2], cerebrosides with extra units of hexose and hexosamine[3] and the above substances with neuraminic acid and/or amino-acids attached[4,5]. The last members of this family, as first isolated[6], were named 'gangliosides'. The addition of these constituents bearing amino-groups is frequently associated with the fact that the complex which is formed bears macromolecular characteristics as determined by non-dialysability and sedimentation values in aqueous ultracentrifugal fields. Although very little is known of the biosynthesis of any of these[7,8], their structural similarities suggest a biosynthetic continuity. Furthermore, attempts to isolate these components by different solvent techniques frequently result in the extraction of protein or lipid substances apparently bound to the aminoglycolipid in either ionic or covalent form, but separable from the aminoglycolipid by a further purification procedure which is almost invariably rather harsh. Structural investigations carried out in this Laboratory[9,10] have led to some clarification of the structure of the macromolecular brain ganglioside of bovine origin and have demonstrated that cerebrosides are clearly structural sub-units of ganglioside[9].

The methods used for the extraction of bovine brain ganglioside have now been applied to human brain specimens obtained at autopsy in 36 mental hospital cases, most of whom were in the eighth and ninth decade of life. Quantitative results were obtained for the yield of 'crude brain ganglioside', neuraminic acid, hexose, hexosamine, nitrogen, and amino-acids, per gm. of whole fresh tissue.

Approximately 100 gm. aliquots of carefully dissected grey and white matter were homogenized for 2 min. with 5 volumes of methanol at room temperature. The homogenate was heated to the boiling point and quickly filtered through a pre-warmed Buchner funnel, the filtrate placed in the cold for 48 hr., the precipitate which formed separated by filtration in the cold and then taken up at room temperature with chloroform–methanol (2 : 1). This 'precipitate fraction' was analysed for dry weight and neuraminic acid, then partitioned as with the preparation of bovine brain ganglioside[10] with distilled water 12 per cent by volume, the upper phase removed and the lower phase again washed with 6 per cent water. The combined upper phases were designated 'crude brain ganglioside', taken to dryness by vacuum distillation at 55–60° C., taken up quantitatively with water and dialysed exhaustively at 4° C. in pre-washed 'Cellophane' bags against a double volume of water. After less than 0·5 mgm. was obtained in the outer water (usually by the tenth change) the dialysed material was collected quantitatively with water washings and analysed for its content of dry weight, nitrogen, neuraminic acid, hexosamine, and hexose[10]. Aliquots of this 'crude brain ganglioside' were hydrolysed with varying concentrations of acid and varying time to determine the maximum yield of hexosamine. This was found constantly to be obtained by 15 hr. of hydrolysis with *N* hydrochloric acid in a sealed tube at 100° C. in 6 hydrolysis curves ($\frac{1}{2}$, 1, 3, 5, 7, 9, 12 15, 24, 36, and 48 hr.) on five different brains. Then hexosamine was determined as a routine in this manner. Another aliquot of 'crude brain ganglioside' was hydrolysed with 6 *N* hydrochloric acid in sealed tubes at 100° C. for 1, 3, 6, 12, 24, and 36 hr. and the hydrolysate was tested quantitatively for free amino-nitrogen[11] and examined by paper chromatography[12]. Semi-quantitative paper chromatography of amino-acids present in this dialysed material after 36 hr. of hydrolysis demonstrated the presence of serine, glycine, glutamic acid, aspartic acid, cysteic acid, arginine, alanine, *iso*leucine, leucine, lysine, and one as yet unidentified amino-acid quantitatively present in approximately the order named. These findings are similar in some respects, but quite different in others from those described for a similar preparation from ox brain[5]. Thus threonine, phenylalanine, histidine, and valine were absent in our preparation, and serine was present in excess of glutamic acid. With short-term (1 and 3 hr.) hydrolyses, some indication was obtained of the order of release of these amino-acids. Glycine and serine were almost completely released in 1 hr., the amounts of arginine, glutamic acid, aspartic acid were present in approximately one-half their total, together with only traces of the other amino-acids mentioned here.

Table 1 shows some of the characteristic values for the carbohydrate constituents obtained to date. It may be noted that the crude brain ganglioside material obtained by identical extraction procedures in different brains analyses differently in each case, not only with regard to the absolute amounts of neuraminic acid, hexose, and hexosamine obtained per gram of whole tissue, but also with regard to their relative ratios. There is a higher content of hexosamine with reference to neuraminic acid in human brain and usually still higher content of hexose. It is therefore clear that this 'crude brain ganglioside fraction' derived from human brain by hot alcoholic extraction contains related non-dialysable aminoglycolipids other than bovine brain ganglioside as classically defined in terms of percentage constituents by Klenk[6] and structurally clarified in our own investigations[10]. Thus, our bovine brain ganglioside contains, in ash-free form, 30 per cent neuraminic acid, 24 per cent hexose and 10 per cent hexosamine, and the salt form of bovine brain ganglioside contains approximately

Table 1. Yield of 'Crude Brain Ganglioside' and Constituent Neuraminic Acid, Hexosamine and Hexose in 36 Individual Human Brain Specimens

	'Crude brain ganglioside' (mgm./gm. whole tissue)	Neuraminic acid (μgm./gm. whole tissue)	Hexosamine (μgm./gm. whole tissue)	Hexose (μgm./gm. whole tissue)	Nitrogen (per cent)
Grey matter					
Mean	2·20	248·3	143·1	247·9	1·54
Range	0·44–4·50	105–406	29–284	54–512	0·5–2·8
White matter					
Mean	2·10	66·0	48·0	179·0	2·2
Range	0·13–7·3	9·7–191	3·2–124	31–419	1·1–5·0

26, 20·8, and 8·7 per cent respectively of these three constituents. In no case so far analysed did these ratios hold for the material examined. There must therefore be either a different type of ganglioside in the human brain, or a ganglioside similar to that found in bovine brain is present, but considerably contaminated by substances richer in hexose and hexosamine which are non-dialysable and partitioned to this point along with the hypothetical human brain ganglioside.

In the purification of bovine brain ganglioside, the crude material is repartitioned four of five times between chloroform - methanol and water[10]. When crude brain ganglioside material, after isolation from brains Nos. 63, 65, 69, 70, 72, was combined and further repartitioned four times, the lower phase contained some 9 or 10 per cent of the dry weight of the fraction at each stage and the material in the lower phase contained increasing amounts of both neuraminic acid and hexose. Thus, the lower phase from the second, third, and fourth repartition contained respectively hexose 6·7, 8·9, 12·4 per cent and neuraminic acid 4·6, 10·8 and 13·0 per cent. The upper-phase material remaining after the fourth repartition now contained 16 per cent hexose and 22·3 per cent neuraminic acid thus approaching the theoretical concentrations and molar ratios of these substances observed in purified brain ganglioside. Further purification and structural investigations on larger quantities of this material are in progress.

It is clear from these results that the classical brain ganglioside is only one of a group of non-dialysable aminoglycolipids which can be extracted from human brain grey matter by means of the hot-methanol method described; that the other substances so extracted are quite rich in hexose, hexosamine, and neuraminic acid; and that these substances can be separated from each other. It would therefore appear that a large family of related substances exists in human brain with varying amounts of the structural constituents described here, in macromolecular association, which might be grouped under the term 'aminoglycolipids'. From a practical point of view, it is clear from our results that neither hexose, hexosamine, neuraminic acid, nor any combination of these three components isolated by the above techniques can be used to extrapolate the concentration of brain ganglioside[12]. The next communication demonstrates that, in addition to the heterogeneity of these materials isolated with alcoholic solvents, the yield in terms of both neuraminic acid and hexosamine is only one-third to one-fifth of the total which can be demonstrated by the emulsion-fractionation procedure developed.

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Peter C. Belval
Dorothy Winer

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3. Quantitative Separation and Determination of Brain Glycoproteins, Neuraminic Acid, Hexosamine, and Hexoses by Means of Whole Tissue Emulsion-Fractionation

The demonstration that there is a family of non-dialysable aminoglycolipids in brain related to classical brain ganglioside[1,2] but differing from it in terms of the relatively greater content of hexosamine and hexose (previous communication) has made it necessary to devise suitable methods whereby all these substances may be extracted quantitatively, further purified, and studied in terms of their relative concentrations in individual brain specimens. In addition, these observations have raised the question of the nature of the macromolecular organization of these constituents *in situ*. This organization would appear to be well worth studying because, on one hand, electron microscopic observations may approach visualization of these complexes and, on the other hand, better understanding of such biological problems as the properties of living cell membranes in terms of receptor and transport functions appear to require better structural definition of what might be called the molecular geometry of membrane components.

Thus, for example, the isolation of brain ganglioside, which has been shown in this Laboratory to have physiological properties suggesting membrane receptor and transport functions[3–6], has generally been accomplished by means of hot or cold organic solvent mixtures. In one method of extraction[1], this aminoglycolipid is obtained in crystalline form and without the presence of amino-acids; in a second method[2], essentially the same glycolipid is obtained with further macromolecular properties, but still without amino-acid constituents; and in a third method of extraction, the glycolipid is obtained with macromolecular characteristics and with the presence of attached amino-acid constituents[7]. These experiments suggest that it would be fruitful to approach these macromolecular aminoglycolipids from the 'protein point of view'. That is, where harsh organic solvents might dissociate protein – amino-glycolipid complexes, leaving only a few amino-acids tied to the aminoglycolipid (previous com-

munication, and ref. 7), solvent methods which are directed towards isolation of the proteins of brain might accomplish the isolation of the intact protein and carry with the protein the glycolipid constituents.

A qualitative method for approaching this problem has been developed in recent work in this Laboratory and will be reported here briefly, and elsewhere more extensively. By means of the emulsion-fractionation method to be described, essentially all the proteins of the brain are separated into nine primary fractions in a manner which does not necessitate the discarding of large amounts of protein as insoluble[5], and which permits further fractionation to be undertaken. Furthermore, new results for the total concentrations of brain neuraminic acid, hexosamine, and hexose are thereby obtained.

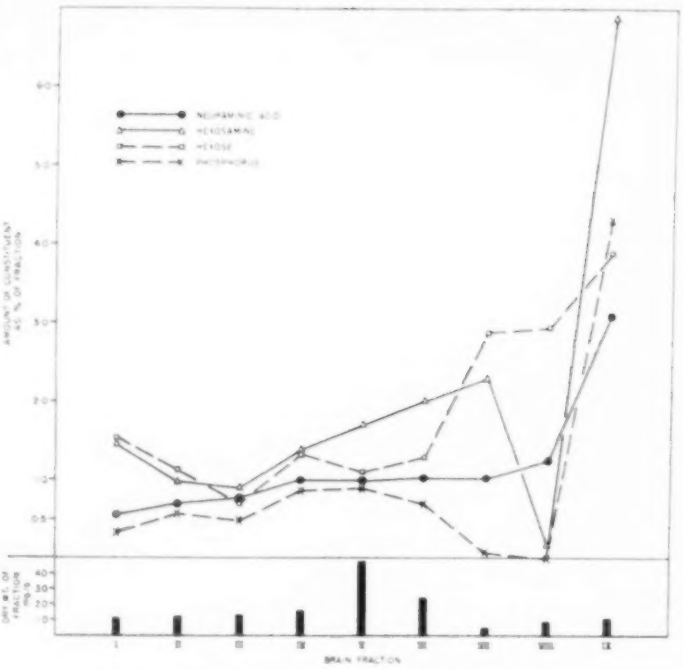

Fig. 1. Emulsion fractionation of human brain grey matter

Freshly autopsied human and bovine brains were immediately stripped of investing meninges and blood vessels and grey and white matter carefully dissected from particular anatomical areas. While the results reported will be on the basis of 5-gm. aliquots of dissected grey matter, all the procedure can be carried out with 1 gm. or less of whole tissue and has been done with white matter as well. 5 gm. of brain tissue is homogenized in a Waring blendor with 180 c.c. of distilled water for exactly 5 min. The temperature at the end of homogenization is 32-33° C. The balance of the procedure is carried out at room temperature (25° C.). (The entire procedure is also effectively carried out at 0°.) The rather stable emulsion which is formed is transferred with 3 c.c. water washing to centrifuge bottles and centrifuged at 1,300 r.p.m. (225 *g*) for exactly 10 min. The precipitate which results is denoted fraction I. The supernatant is carefully decanted, methanol is added to the extent of 50 per cent by volume, the solution is mixed and immediately centrifuged for exactly 10 min. The precipitate which results is denoted fraction II. To the supernatant, which has been carefully decanted, is added sufficient methanol to reach an equivalent of one volume relative to the original volume of emulsion. The solution is immediately centrifuged as above and the precipitate (fraction III) is obtained. By successive addition to the supernatant at each stage of aliquots of methanol so that the final solution contains 1·5, 2, 2·5, 3, and 3·5 volumes of methanol relative to the original volume of emulsion, precipitates are obtained at each stage which are successively denoted fractions IV – VIII. The supernatant remaining after precipitation of fraction VIII with 3·5 volumes of methanol, which is now water-clear and which no longer yields appreciable further precipitate on the addition of further volumes of methanol, is taken to dryness by vacuum distillation at 55–60° C. and is designated fraction IX. Each of these nine fractions is quantitatively taken up in distilled water to form a 1–10 mgm. per c.c. suspension. This suspension can then be analysed, taken up in buffers, or further partitioned into dialysable and non-dialysable constituents. For this further partition the suspension is transferred quantitatively with distilled water to pre-washed 'Cellophane' bags and dialysed at 4° C. against equal volumes of distilled water if collection and further study of the dialysable material is desirable, or against 10- to 20-fold volumes of distilled water for more rapid isolation of non-dialysable components when the dialysable components are not sought.

Fig. 1 summarizes a typical emulsion-fractionation procedure in terms of 5 gm. of grey matter. The dry weight is shown in the bar graph and the percentage of phosphorus, neuraminic acid, hexose, and hexosamine is shown for each of the nine dialysed fractions. The analytical methods used are as given in the previous two communications. It may be seen that each of the nine fractions is distinguished not only in terms of its precipitability at different concentrations of methanol, but also in terms of its percentage composition of the above constituents. Thus, the early fractions are poor in phospholipid, relatively rich in bound hexose, and relatively poor in bound neuraminic acid. With increasing concentration of methanol, non-dialysable complexes are precipitated with varying, but generally increasing concentrations of neuraminic acid, hexosamine, and hexose. The concentrations of nitrogen in fractions I–IX respectively are 4·6, 5·2, 4·8, 13·2, 8·1, 6·8, 6·1, 5·2 and 14·2 per cent. The concentration of bound hexose increases markedly in the last three fractions. Phosphorus is low in fractions VII and VIII, and the concentrations of nitrogen, hexose, hexosamine, and neuraminic acid in fractions

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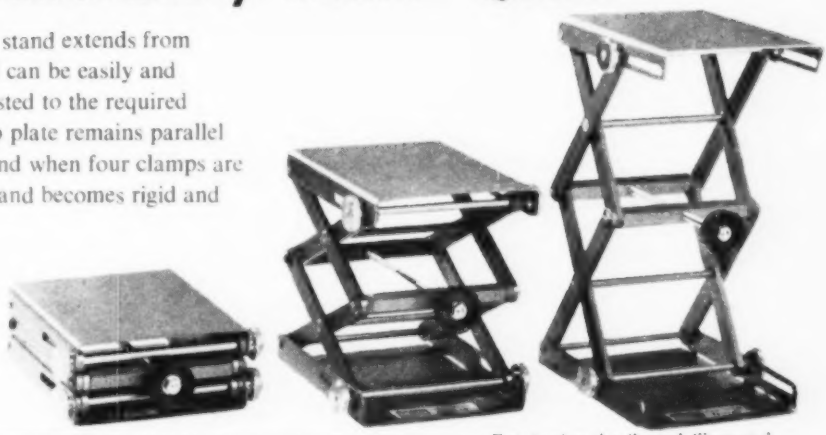

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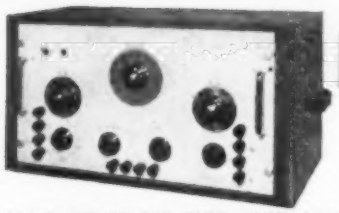

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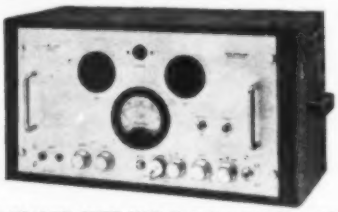

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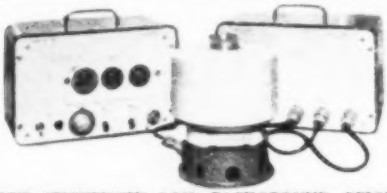

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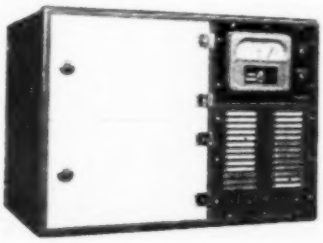

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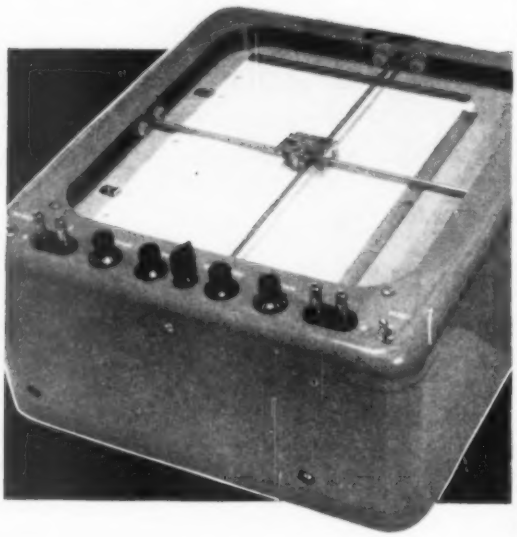

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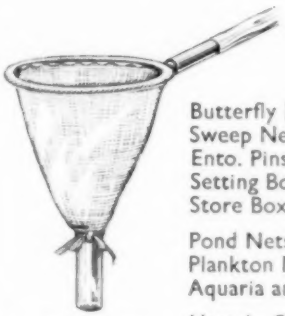

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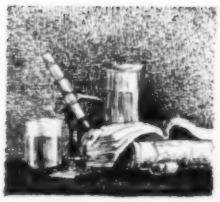

IN APRIL—(1799)—Medical and Physical Journal (London) reviews[1] an essay by Citizen Fourcroy on a newly discovered and controversial substance called "oxygen"—the very name of which, he feels, will draw upon him "numerous groups of men, animated by different opinions and passions, but alike enemies to this [concept]." Fourcroy believes that the animal system may be impaired either by excess or deficiency of this important vivefying principle. Its use, either internally or externally in particular diseases excites the "action of life". Therefore, remedies may be classified as oxygenating or deoxygenating. The former increase the activity of the whole system, heat, circulation, force, and motion; the latter, on the contrary, diminish all these natural effects.

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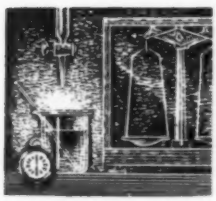

IN APRIL—(1925)—Harvard investigators report[2] their estimations of the minimum molecular weight of 14 proteins. They point out, however, that present analytical techniques do not permit placing too great confidence in any single set of determinations. In some instances (e.g., the histidine and the tryptophane content of casein) different procedures have yielded significantly different results. "Only when different methods have led different investigators to essentially the same conclusion can we proceed to the furthest implications of the result."

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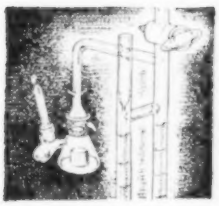

IN APRIL—(1949)—There is described[3] the preparation of a stable beef brain powder with good hexokinase and phosphohexokinase activity. This powder phosphorylates glucose, fructose, mannose, and their 6-phosphates, but not galactose, ribose, or gluconic acid. Sodium salts inhibit phosphorylation of glucose, but not of fructose or fructose-6-phosphate. The ratio of glucose to fructose-phosphorylating activity varies in different brain extracts. It is suggested hereby that there exist two separate hexokinases which are specific for glucose and fructose.

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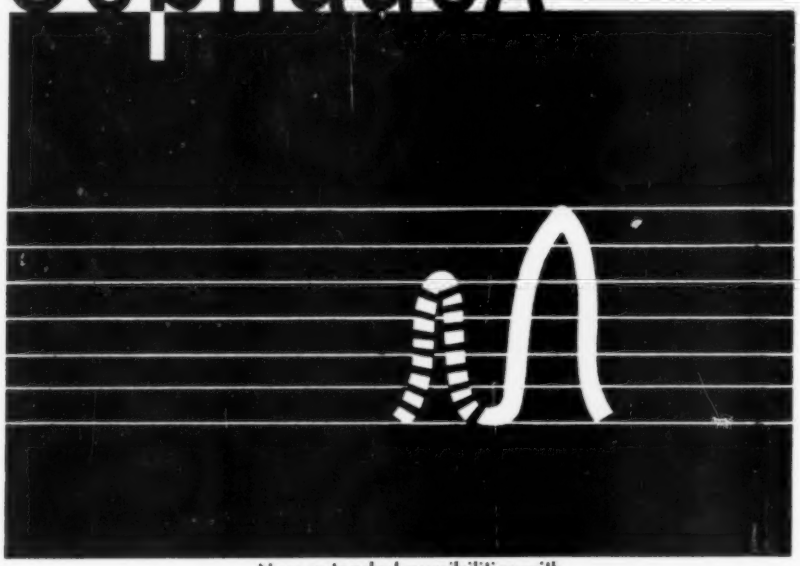

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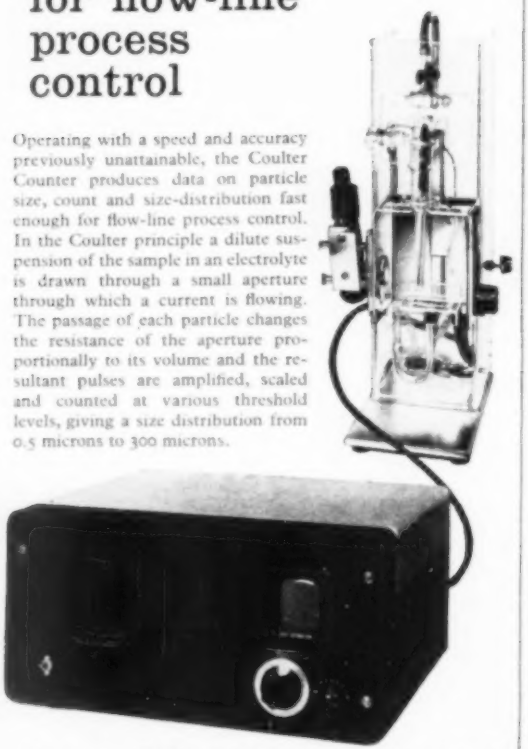

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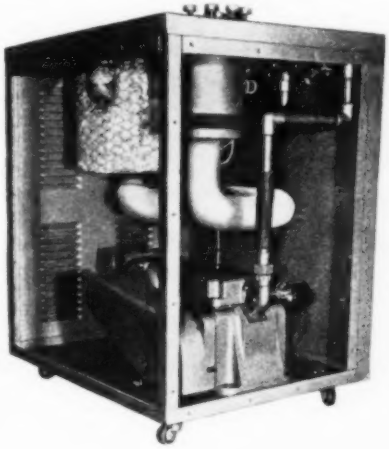

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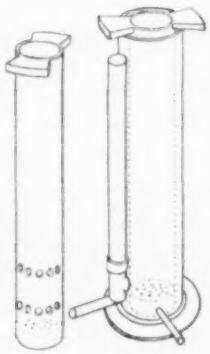

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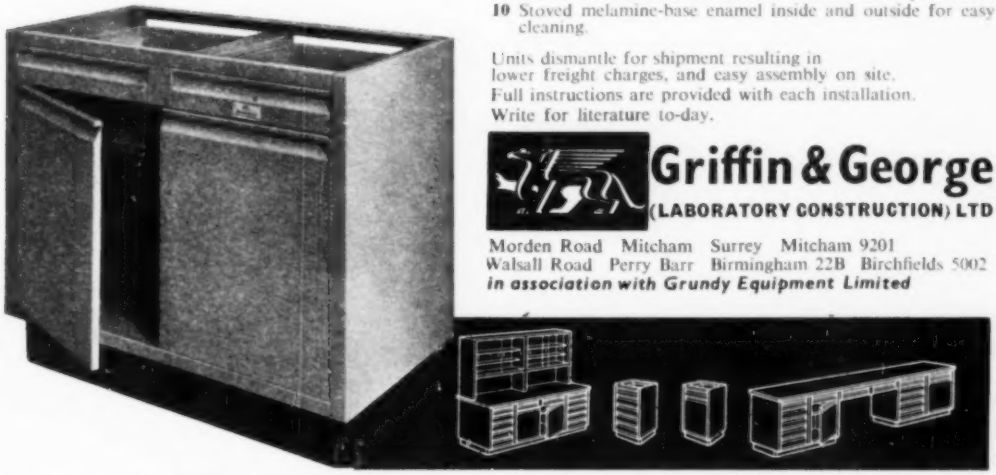

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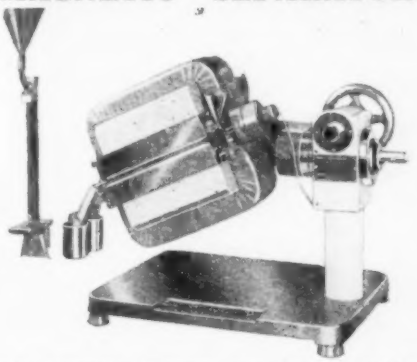

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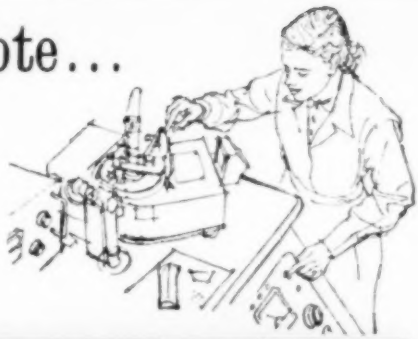

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Each of these fractions can be fractionated further by virtue of solubility in different buffer systems or, as illustrated here, by treatment with mixed solvent systems. Thus, for example, in one brain when fraction IX was partitioned between the three-solvent system of chloroform–water–methanol[8], whereas the lower phase contains lipid material with only 3·6 per cent nitrogen, 0·19 per cent neuraminic acid, and 1·6 per cent hexose, the first two upper phases which are obtained combined contain glycoprotein CIX*a* with a nitrogen content of 11·8 per cent, neuraminic acid content of 1·7 per cent and hexose content of 4·7 per cent. In other brain specimens CIX*a* contained as much as 4·1 per cent neuraminic acid and 8·0 per cent hexose.

The above emulsion-fractionation method thus permits the quantitative fractionation of all brain proteins into nine primary fractions in association with native lipids in the form of emulsions (not true solutions, with the exception of fraction IX), in which the protein components appear to be protected from gross denaturation by the presence of the associated lipids (possibly the same lipids as those *in situ*) and by the absence of harsh organic solvents at the preliminary separation stages. This procedure, in contrast to others[8], does not produce masses of insoluble denatured protein amounting to 50–90 per cent of the total protein content of brain.

The yield of grey matter neuraminic acid by emulsion-fractionation (1·7–1·8 mgm./gm. whole tissue) is in excess of that obtained with chloroform–methanol[10] and with hot methanol techniques (previous communication). The partition of fractions CIV, CV and CIX by chloroform–methanol–water demonstrates that much of the neuraminic acid of brain previously assumed to be part of brain ganglioside is in fact part of other aminoglycolipids and glycoproteins.

Similarly, the yield of hexosamine from human grey matter alone by emulsion-fractionation (2·3–2·7 mgm./gm. fresh tissue) is two or three times that previously reported[12] for whole bovine brain. In addition to higher total yield, the disadvantage of the multiple organic solvent and water extractions for hexosamine[12] (which left 62 per cent of the recovered brain hexosamine in the insoluble residue) is overcome.

Both hexosamine and neuraminic acid are seen here in association with several brain glycoprotein fractions, which vary in the ratios of their content of each of these constituents. The total concentration of grey matter hexosamine is found to be in excess of that of neuraminic acid (whereas in purified brain ganglioside the reverse is true, that is, neuraminic acid being present in threefold excess of hexosamine by weight). Therefore, attempts to estimate brain ganglioside on the basis of the amounts of total neuraminic acid and/or hexosamine which can be extracted[11,13] cannot be considered valid, and brain ganglioside can only be determined at present by isolation of the material which has the characteristic ratios of these constituents. The presence of this non-ganglioside brain hexosamine and neuraminic acid is partially accounted for by brain glycoproteins.

The yield of grey matter hexose by emulsion-fractionation is 2·2–6·0 mgm./gm. fresh tissue. Whereas essentially all the neuraminic acid and hexosamine is non-dialysable, 16–40 per cent of grey matter hexose is dialysable, that is, approximately 1 mgm./gm. fresh tissue, a figure in good agreement with those given previously for 'labile' brain glucose[9].

There is evidence of variation between specimens of brain from different individuals in the total amounts of each constituent in different fractions, a finding of interest in relation to the high degree of chemical individuality observed in this Laboratory in work on the same constituents in another compartment of the nervous system, namely, the glycoproteins of human cerebrospinal fluid[14–16].

The further isolation of individual brain glycoprotein components from each of the nine primary fractions is now in progress and those are denoted CI*a*, CI*b*, etc. for cortical grey matter glycoproteins, and WI*a*, WI*b*, etc. for white matter glycoproteins. Antisera have been prepared in rabbits to fractions CIV and CV and the histological localization of these substances observed in human brain sections by the fluorescent antibody technique, as done in the case of brain gangliosides[4]. Preliminary results suggest a predominantly glial localization of CV and a predominantly neuronal localization of CIV, and the method is being extended to the other seven fractions.

The emulsion fractionation method described provides a simpler, yet more quantitative means of estimating brain neuraminic acid, hexosamine, and hexose in both normal and pathological specimens of brain than methods previously available; permits the quantitative separation and study of nine primary individual brain glycoprotein fractions which differ in their chemical composition; and permits the further separation of sub-fractions from each of the primary ones. In addition, because of the relative mildness of the procedure, it offers an opportunity for enzymatic studies on one hand, and for histological studies on the other, and therefore may be useful in approaching structure-function problems with reference to glycoproteins and aminoglycolipids in the nervous system.

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LETTERS TO THE EDITORS

ASTROPHYSICS

Terrestrial Accretion from the Solar Wind

In order to account for the observed motions of comet tails, Biermann[1] conceived that the solar corona emits suprathermal particles at the rate of 10^{14}–10^{15} gm./sec. Since this material is evidently supplied by the Sun itself, this seems to imply that Unno's observation[2] of turbulent motions in the chromosphere and lower corona corresponds in reality to a systematic radial emission of particles from the Sun, the velocity v of which is related to the hydrogen ion concentration n by the relation $v = Bn^{-1/4}$, where $B = 8 \times 10^8$ (cm.)$^{1/4}$ sec.$^{-1}$. This empirical relation is of the same form as Parker's magneto-hydrodynamical velocity-amplitude relation[3,4], $v(r) = v_0(n_0/n_r)^{1/4}$, the applicability of which is certainly not restricted to the chromosphere. Thus the possibility of extrapolating Unno's result to interplanetary space deserves further consideration.

Application of the equation of continuity to this flux yields nr^2v = constant, r being the radial distance from the centre of the Sun. Combining this with Unno's relation, one obtains $n \propto r^{-8/3}$ and $v = Kr^{2/3}$, where K is a constant. Writing $v = dr/dt$ and integrating the resulting equation, the transit time of a proton in the solar wind from the solar surface at r_0 to the orbit of the Earth at r_1 is $t = 3(r_1^{1/3} - r_0^{1/3})/K$, and since $K = v_1/r_1^{2/3}$, we obtain:

$$v_1 = \frac{3r_1}{t}\left[1 - \left(\frac{r_0}{r_1}\right)^{1/3}\right]$$

Transit time measurements show that t is about one day, hence $v_1 = 4{\cdot}33 \times 10^8$ cm./sec. and $n_1 = (B/v_1)^4 = 11{\cdot}65$ protons/cm.^{3} at the orbit of the Earth.

This result is compatible with the recent findings of Popo[5] (from a study of whistlers) and of Kupperian[6] (from Lyman α observations). It is also reasonably compatible with van de Hulst's estimate of 3×10^7 electrons/cm.^{3} at a distance $r_c = 10^{11}$ cm. from the centre of the Sun, for which the present theory gives a concentration $n_c = n_1(r_1/r_c)^{8/3} = 0{\cdot}74 \times 10^7$ protons/cm.^{3}. On the other hand, Biermann suggests that at the orbit of the Earth, $n_1 = 500$ protons/cm.^{3} on magnetically quiet days, and quotes the outward radial velocity v_1 as ranging from 500 to 1,500 km./sec. It would seem that Biermann's n_1 is too high, his v_1 too low; the same dynamical effects on comet tails, dependent on $n_1v_1^2$, would result from the extrapolation of Unno's relation. Denoting the proton mass by m_H, the rate of emission of hydrogen by the Sun is then $4\pi r_1^2 n_1 m_H v_1 = 2{\cdot}4 \times 10^{12}$ gm./sec.

Moreover, the energy of a proton travelling at $4{\cdot}3 \times 10^8$ cm./sec. is about 100 keV., and assuming a localized equipartition of proton and electron energy within the outer Van Allen radiation belt, the mean energy of the electrons in this region must be 50 keV., in accord with Van Allen's results from satellite data. Electrons of this energy have a velocity $v = 1{\cdot}24 \times 10^{10}$ cm./sec., yielding an electron flux $n_1v = 1{\cdot}44 \times 10^{11}$/cm.^{2} sec., again in accord with recent observations.

The rate of accretion of hydrogen by the Earth from the solar wind is of great interest. The Earth must have been exposed to this flux throughout its estimated age, $T = 3{\cdot}3 \times 10^9$ years or $1{\cdot}04 \times 10^{17}$ sec. Assuming that the magnetic field of the Earth remains effective in capturing protons and electrons up to a distance $r_m = 12{\cdot}3$ Earth radii from the Earth's centre, as suggested by the onset of field disturbances at this distance[5], the total mass of hydrogen accreted by the Earth throughout its existence is $\pi r_m^2 n_1 m_H v_1 T = 1{\cdot}70 \times 10^{22}$ gm. Complete oxidation of this hydrogen would yield $1{\cdot}53 \times 10^{23}$ gm. of water, in close accord with the known ocean mass[6] of $1{\cdot}42 \times 10^{24}$ gm. This remarkable result indicates that the seas must have been formed gradually by accretion of solar hydrogen at the rate of about 1·5 tons/sec., and that this process is still taking place.

Finally, the presence of about 1 part of deuterium in 6,700 of hydrogen in sea-water is in reasonable agreement with Severnyi's estimate[7] of the relative abundance of deuterium at the solar surface, especially when allowance is made for the possibility of isotopic fractionation during the transfer process. This implies in turn a rate of emission of deuterium of $3{\cdot}5 \times 10^{10}$ deuterium atoms/cm.^{2} sec. from the solar surface. The question of the origin of this deuterium remains one of the greatest unsolved problems of solar physics.

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GEOPHYSICS

Measurement of Positive-Ion Density in the Ionosphere by Sounding Rocket

Two sounding rockets, *Kappa*-VIII-3 and -4, a detailed account of which will be published elsewhere, were fired at 15h. 32m. 00s. September 22 and 20h. 25m. 00s. September 26, 1960, in order to observe the vertical profiles of positive ion density in the ionosphere. The launching site was the Akita Rocket Range in Japan, of which the geographical co-ordinates are lat. 39° 34′ N. and long. 140° 04′ E. Simultaneous observation of the ionosphere in vertical incidence was made at the Akita Radio Wave Observatory of the Radio Research Laboratories, geographical co-ordinates lat. 39° 44′ N. and long. 140° 08′ E.

Each rocket was provided with the spherical Langmuir probe in the nose cone, which was split vertically into two parts at an altitude of about 60 km. to expose the ion probe to space.

The probe installed in the rocket for the day-time flight was a meshed spherical cage of molybdenum

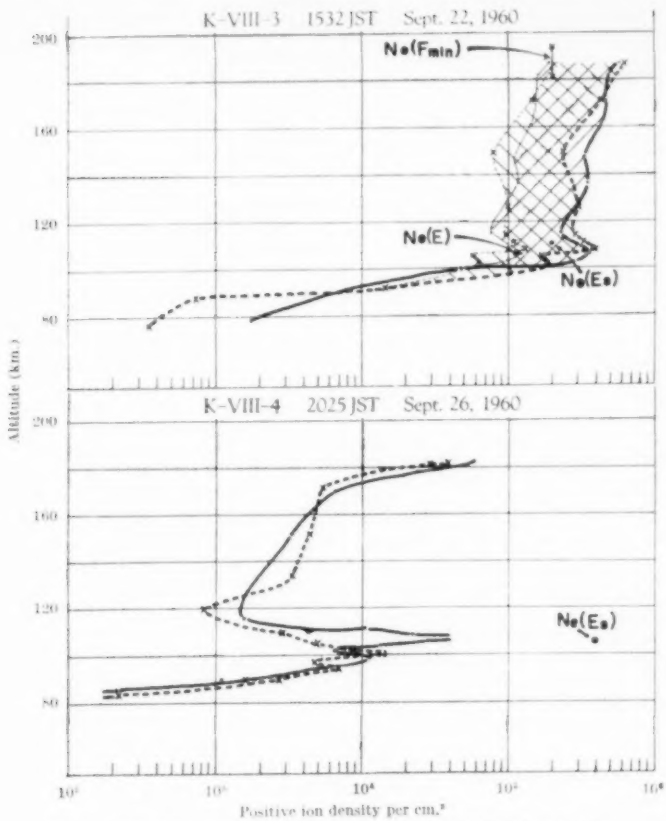

Fig. 1. Plots of ion density as a function of altitude. Observations during ascent, ●—●; during descent, ×---×. $Ne(E)$ is the electron density of E-layer; $Ne(Es)$ that of Es-layer and $Ne(F\text{min.})$ is that of lowest part of F layer. These values are calculated from the ionograms shown in Fig. 2

wire of 0·3 mm. diameter and welded electrically. This form of probe was used so as to eliminate the effect of the strong photo-electron emission from the surface of the probe excited by the ultra-violet component of solar radiation in the upper atmosphere. This effect has been found to cause serious disturbance in the measurement of ion current[1]. The spacing of the wires was such that the ion sheath formed around the probe was perfectly spherical, and observations in the region of low ion density in the ionosphere were reliable. The probe has a diameter of 2·0 cm. and is gold-plated. The probe for the night-time flight was the usual gold-plated spherical shell but of the same diameter.

Each probe was fastened at the top of a long tapered metal rod of 65 cm. to avoid deformation of the ion sheath around the probe due to the rocket body. The metal rod, made of duralumin, was coated with silicone resin to insulate the probe electrically from the environment. When the probe system was immersed in plasma, the ion current in the probe system would flow only from the spherical probe, and calculations of the ion density would be quite trustworthy.

The flights were successful and complete records were obtained throughout both ascent and descent. In this communication, we describe preliminary results analysed from measurements of ion densities by sounding rocket. A complete analysis of the observations will be reported elsewhere.

Fig. 1 shows the profiles of the positive ion density calculated from the observed probe currents at a probe potential of about 20 V. against the body of the rocket. The values of electron temperature and ion mass in the ionosphere, which are essential for calculation of the ion density from the probe current, were taken from Yonezawa's paper[2]. Depending on the behaviour of ions in the ion sheath, different methods of calculation were adopted for various altitudes of the rocket, namely, regions of many collisions, one collision and orbital motion in the sheath. In the orbital region, only a portion of the ions gathered on the probe can collide with the meshed spherical cage. The minimum correction factor, that is, collection probability, is determined here from the geometrical configuration of the probe; the maximum correction factor is believed to be

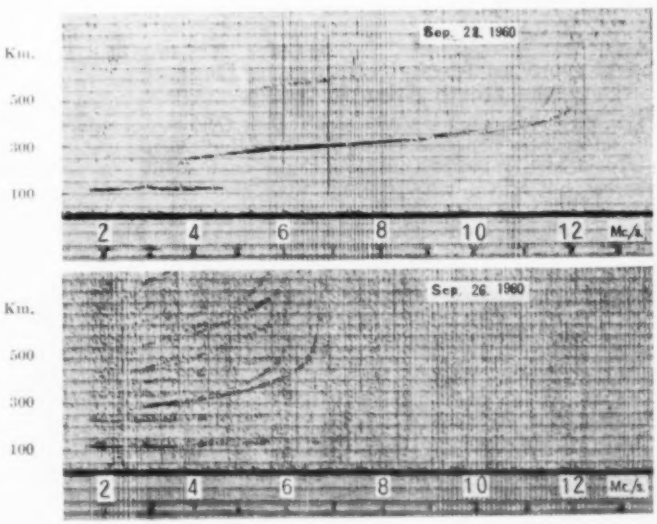

Fig. 2. Ionograms obtained at the Akita Radio Wave Observatory

about three times as great. Hence the results shown by the hatched area in Fig. 1 indicate the probable maximum values of the ion density.

The h'–f curves obtained by vertical incidence observation of the ionosphere are shown in Fig. 2. Numerical values of the electron density at the true height scaled from these h'–f curves are plotted in Fig. 1 in order to compare the ion and electron densities.

It is remarkable that the records both in the daytime and at night during the ascents show close agreement with those during the descents. Also, the numerical values of electron density scaled from h' f records agree fairly well with those for ion density in the measurements by sounding rocket. It is interesting that the night measurements indicate remarkably thin layers of ion density in space of very low density, corresponding to the sporadic E layer observed in h'–f observations. Furthermore, it is noticeable that ions exist from the rather low altitude of 60 km. in the ionosphere during day-time.

We are indebted to the Institute of Industrial Science in the University of Tokyo for firing the sounding rocket of *Kappa* VIII, and we thank Prof. K. Maeda for his encouragement.

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Lavas from an Abyssal Hill on the Floor of the North Atlantic Ocean

In May 1958, during a cruise of R.R.S. *Discovery II*, underwater photographs by Dr. A. S. Laughton revealed rock outcrops and talus slopes on a small abyssal hill which rises 200 m. from the Iberia Abyssal Plain to a summit depth of 4,960 m. and is situated 300 miles north-west of Lisbon at 41° 21′ N., 14° 28′ W. Dredging produced a large collection of rocks from the hill. This collection is significant because it is believed to be unique[1] and may represent material from an outcrop of Hill's "seismic Layer 2"[2]. The petrographic homogeneity of the collection indicates that the dredged stones were not erratics: four are bedded palagonitic vitric-tuffs and four are granitic erratics, but all the remaining three hundred and fifty-four are highly altered vesicular basalt. Some characteristically shaped fragments of pillow lavas contain relatively fresh material, including a few grams of a tachylitic margin which has escaped palagonitization. The magma appears to have been a porphyritic bytownite-rich augite-basalt without olivine. Extensive alteration, believed

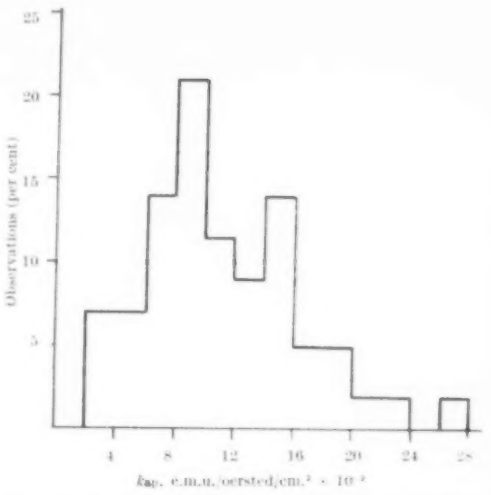

Fig. 1. Histogram showing distribution of apparent susceptibility, $k_{ap.} = \frac{I - kH}{H}$, in 43 specimens of lava. Number of observations (per cent) falling in intervals of 2×10^{-3}. I is intensity of remanent magnetization, k the susceptibility, and H is 0·437 oersted

hydrothermal, has produced relatively soft rocks from the original lavas. In the course of alteration there was significant oxidation of iron; loss of silica, lime and magnesia; gain in potash; manganous oxide was redistributed as pelagite ('manganese') within the rocks. Autoradiographs show that this secondary pelagite is quite strongly radioactive and accounts for the unexpectedly high radioactivity (13 p.p.m. equivalent-U_3O_8, an amount that would be normal for a granite) measured in bulk samples of the altered lavas. The 'manganese' encrusting the stones (visible in Fig. 3) is also quite strongly radioactive (about 50 p.p.m. e-U_3O_8). Probably, both the pelagite within the stones and that in the encrustation were deposited after solution in seawater and acquired its radioactivity from the sea. The available evidence does not suggest that the original basaltic magma was unduly radioactive.

Data presented in Fig. 1 indicate that the altered lavas are slightly less magnetic than normal basalts although their susceptibility (median value 5×10^{-4} e.m.u./oersted/cm.^{3}) and remanent magnetization (median 5×10^{-3} e.m.u./cm.^{3}) lie within the known range of variation for basalts.

Fig. 2 shows results of determinations of compressional wave velocity in the altered lavas. Velocities were measured at room temperature and pressure, parallel to a uniaxial compressive stress of a few tens of atmospheres. Variation of velocity with hydrostatic compacting pressure was measured in six jacketed saturated specimens under differential confining pressures of 1–1,000 kgm./cm.^{2} using apparatus similar to that of Wylie *et al.*[3]. At 1,000 kgm./cm.^{2} the three softer specimens had become crushed and compaction was complete in the others. The maximum increase in velocity observed was 30 per cent. This result, and those of Fig. 2, indicate that the seismic velocity obtaining in these altered lavas under the sea bed would be in, or below, the lower part of the range of velocities (4–6 km./sec.) ascribed to Layer 2. It is suggested that lava flows

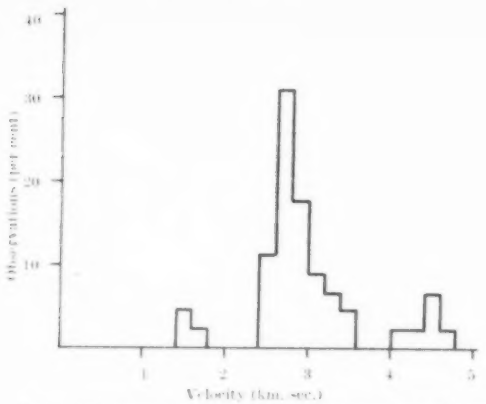

Fig. 2. Histogram showing distribution of compressional wave velocities measured in screw press in 45 water-saturated cylinders of lava. Number of observations (per cent) in intervals of 0·2 km./sec.

of *altered* basalt could be common in Layer 2 under the oceans.

The collection includes twenty large slabs thought to be pieces of the brecciated surface of an outcrop, held together by encrusting 'manganese'. Examination of these slabs (Fig. 3) has suggested details of a weathering process whereby the angular detritus frequently photographed on submarine talus slopes could have been detached from the outcrops. The breccia consists of closely interlocking angular fragments of lava, themselves riddled with cracks lined with dendritic films of pelagite. The wider cracks contain a 'soil' of the clay mineral alteration-products of palagonite and chlorophaeite, with occasional calcite and zeolites. It is suggested that the change in volume associated with the formation of these minerals may serve to prise off angular fragments along the line of pre-existing shrinkage cracks.

Grateful acknowledgment is made to Dr. J. C. Swallow and the Director of the National Institute of Oceanography for facilities in R.R.S. *Discovery II* and to Dr. Bowie of the Geological Survey (Atomic Energy Section) for bulk radiometric assays. Part of this work was supported by a studentship from British Petroleum and part by the U.S. Office of Naval Research under a contract with the University of Cambridge.

Fig. 3. Photograph of sawn section of a slab of 'manganese'-cemented breccia

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PHYSICS

An Apparent Paradox in Crystal Structure Analysis

A commonly used test for the correctness of a crystal structure deduced from X-ray diffraction data is to perform a Fourier synthesis using the observed structure amplitudes ($|F_o|$) and the calculated phases (α_c) in the Fourier coefficients (namely $|F_o|\exp i\alpha_c$) and to verify that this diagram gives peaks of the right magnitudes at the assumed positions of atoms and none elsewhere. It was thought worth while examining what would happen if the amplitudes and the phases that are fed in belong to different structures. The tests that were carried out are briefly described here, together with an explanation of the results observed and their relation to the methods of structure analysis.

A series of syntheses were first calculated using the phases (α_c) calculated from the atomic positions of tyrosine hydrochloride[1], but employing (*a*) structure amplitudes that were completely random, (*b*) structure amplitudes which were made equal for all reflexions except for a falling off with ($\sin\theta/\lambda$) corresponding to the unitary structure factor and (*c*) using a set of amplitudes which were made, so far as possible, anti-correlated to the correct values about the mean. In every case, the atomic peaks occurred at the same positions as in the assumed structure from which the phases were calculated.

This result, quantitative details of which will be published elsewhere, confirms the known fact that, particularly for a non-centro-symmetric structure, correct phases are of much greater importance than correct amplitudes in determining the positional co-ordinates of atoms[2]. For example, severe extinction can be tolerated provided that only atomic positions are required.

These tests suggested another possibility, namely, the use of the calculated phases of one crystal and the structure amplitudes of a completely different crystal and seeing what the resultant Fourier synthesis would look like. Fig. 1 (*a*) was obtained in this way. It is the *c* projection of a crystal (symmetry $P2_1$) for which the phases were obtained using the positions of the 13 light atoms in the molecule of tyrosine (as it occurs in tyrosine hydrochloride[1], $a = 11{\cdot}1$ Å., $b = 9{\cdot}0$ Å., $c = 5{\cdot}1$ Å., $\beta = 92°$, $Z = 2$, but without the two chlorine atoms) and the structure amplitudes $|F_o|$ of an imaginary crystal having 6 atoms in its asymmetric unit, but having the same unit cell and the same number of electrons in the unit cell. The positions of these atoms are marked by crosses in Fig. 1. They were taken to have a suitable scattering factor falling off with angle as for a carbon atom, so that the average intensity for both crystals would be

exactly the same. Fig. 1 (*b*) is the electron-density diagram obtained by feeding back the data for tyrosine, that is, using both the amplitudes $|F_c|$ and the phases α_c of tyrosine itself. It will be noticed that there is a marked agreement between the two diagrams, and that all the atoms of tyrosine have come out in the first diagram even though the data for structure amplitudes used were of an entirely different crystal. The peak heights which are equal in Fig. 1 (*b*) are, however, very unequal in Fig. 1 (*a*) and traces of new peaks have appeared, one of which is about equal in electron density to one of the tyrosine atoms. Nevertheless, the absence of any semblance of a peak at three of the atomic positions of the structure amplitude data of the crystal used (marked by crosses) is very striking.

The use of a 'difference synthesis' cannot be expected to reveal the atomic positions of these three missing but 'correct' atoms, since for a difference synthesis to be meaningful, F_o and F_c must be very nearly equal in phase. However, Fig. 2 has been calculated using $(|F_o| - |F_c|) \exp i\alpha_c$ as the Fourier coefficients, where $|F_o|$ refers to the six-atom structure and $|F_c|$ to tyrosine. It will be noticed that although, at some of the tyrosine atoms (marked by dots), there is a small negative density, and although three of the X positions still indicate a very small positive density, the other three X atoms still fail to appear. In general, the positive and negative densities do not exceed that of a helium atom anywhere in this diagram.

It appears to be a logical conclusion to make from the above results that, if a Fourier synthesis is made using the amplitudes of the correct structure, but the phases calculated from assumed atomic positions

Fig. 1 (*a*)

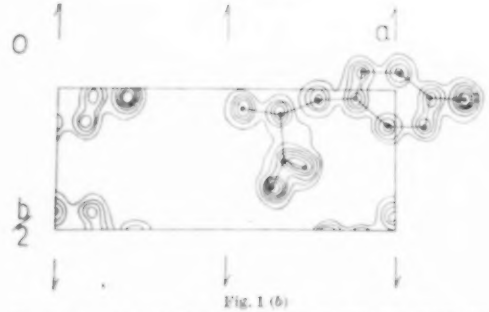

Fig. 1 (*b*)

Fig. 1. *c*-axis projection of the Fourier syntheses using the coefficients (*a*) $|F_o| \exp i\alpha_c$; (*b*) $|F_c| \exp i\alpha_c$, where *o* refers to a hypothetical six-atom structure marked by crosses and *c* refers to the structure of L-tyrosine. Contours are at intervals of 1 e./Å.^{3}, starting from 1 e./Å.^{3}

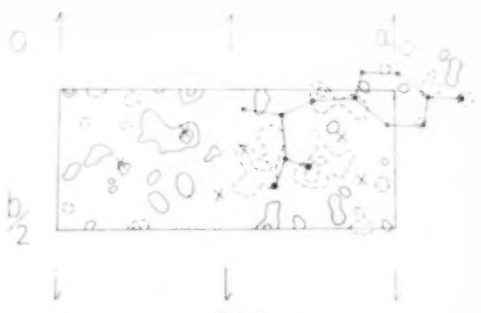

Fig. 2

Fig. 2. The difference Fourier synthesis $(|F_o| - |F_c|) \exp i\alpha_c$. Contours are at intervals of 1 e./Å.^{3}. Positive contours are shown by continuous lines and negative by broken lines. The zero contour is omitted

different from these, then the resultant synthesis would contain peaks at the assumed positions, but not necessarily at the correct atomic positions. On the other hand, it is well known that the method of using a series of successive Fourier syntheses often gives the correct structure and in fact converges to it, which is in apparent contradiction with the above statement.

There is, however, no real contradiction if it is remembered that in the present test the assumed atomic positions are *all* completely wrong; whereas, in structure analyses which converge, at least some of the atoms of the assumed structure would be at the correct positions (for example, the heavy atoms when such are present). The difference between the two cases is seen most strikingly by applying the general ideas of the de-convolution of the Patterson function and of the modulation of one structure by another developed in our laboratory[3,4]. Thus, the synthesis $|F_A| \exp i\alpha_B$ (where $|F_A|$ is the amplitude corresponding to a structure A and α_B is the phase corresponding to a structure B) is essentially equivalent to the modulations of the Patterson diagram of the structure A with the actual structure B. In this, it is not difficult to show that peaks occur only corresponding to the atomic positions of B and none of A, provided that A and B have no atoms in common. However, if the structures A and B have some atoms (P) in common, then not only would these atoms (P) and the remaining atoms of B come out prominently, but also there would be weaker peaks at the positions of the remaining atoms of A also, their strength being greater the larger the ratio $\sigma_P^2 = \sum_1^P f_j^2 \Big/ \sum_1^A f_j^2$. This last result shows that a necessary condition for peaks to occur at the unknown 'correct' atomic positions is that at least some of the atoms used for calculating the phases must be at correct or nearly correct positions. Since it is the mean contribution to the intensity of these correct atoms that determines the strength of the peaks at the unknown atomic positions, a small number of heavy atoms would serve this purpose equally well. Looked at from the phase point of view, convergence is usually fast in successive refinements in the heavy-atom technique because a large proportion of the phases is already nearly correct at the beginning. In fact, if the value of σ_P^2 is 50 per cent (or about half the phases are correct), a structure would refine rapidly even from very high R values.

As mentioned earlier, a peak has actually appeared at one of the crosses in Fig. 1 (*a*), contrary to the theoretical prediction. We believe this is fortuitous, and this is confirmed by the fact that no peaks at all have developed either in this figure or in the 'difference synthesis' at three other such positions.

It will also be noticed that in Fig. 1 (*a*), the peaks in the assumed positions have re-appeared with unequal peak heights, which itself would indicate that it is not correct, in addition to the large value that occurs for the residual factor (R value).

There has been some discussion in the literature (see, for example, ref. 5) as to whether the R value is really useful in judging the degree of correctness of an assumed structure, particularly since some structures with fairly low R values have turned out to be incorrect. The difficulty in the past has been that experimental errors were often greater than 10 per cent in $F_{obs.}$, and it was sometimes difficult to distinguish between errors arising from this cause and those arising from a part of the assumed structure being wrong. So also, in some cases, one projection would refine well whereas the others would not, because one set of positional co-ordinates was wrong or because of a wrong choice of origin out of two possibilities. The occurrence of disorder in one direction or of a very anisotropic temperature factor would also lead to difficulties. However, if three-dimensional data are used and an accuracy of better than 10 per cent in $F_{obs.}$ is attained, then the probability of the R value being low and yet the structure being wrong would be negligible.

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Electroluminescence of Gallium Phosphide Crystals

In the first papers on the electroluminescence of gallium phosphide[1], photographs of the emission spectra of undoped and zinc-doped crystals were observed to differ. As a working hypothesis, Wolff, Hebert and Broder[1] suggested that zinc might form a luminescent centre in gallium phosphide analogous to copper in zinc sulphide. Carrying this analogy further, they proposed that the zinc centres are ionized by impact with charge carriers injected at the negative electrode (the luminescence in most cases originated at the negative electrode). Since this work was reported, several workers[2–5] have observed electroluminescence at grain boundaries in gallium phosphide polycrystals and have proposed p–n junction recombination to be the dominant luminescent mechanism. Grimmeiss and Koelmans[2] report that undoped or weakly zinc-doped electroluminescing polycrystals can be shown to contain both p- and n-type regions (they were mainly n-type) and all give the same emission spectrum. Heavily zinc-doped samples were uniformly p-type and did not electroluminesce. I have investigated the electroluminescence of gallium phosphide single crystals of uniform conductivity type and find the emission spectrum from undoped n-type crystals to be similar to that reported by Grimmeiss and Koelmans. Weakly zinc-doped crystals, however, are p-type (10^{17} carriers/c.c., 50 cm.^{2}/V. sec. mobility, from Hall effect measurements) and give a unique electroluminescence emission which is probably the same as that observed by Wolff *et al.* (The 'zinc' electroluminescence has also been observed by M. Gershenzon (private communication).) Heavily zinc-doped crystals are darker in colour and do not electroluminesce, in accord with the observations of Grimmeiss and Koelmans.

The crystals studied were grown from solutions of 10–20 atomic per cent of phosphorus in gallium by slow cooling from about 1,200° C. The silica vial was in a constant-temperature zone of the furnace throughout the crystal-growth operation. Zinc was introduced by mixing metallic zinc with the gallium and phosphorus before heating. The crystals obtained from these procedures are usually platelets with <111> faces and dimensions of about 5 × 2 × 0·5 mm. They are single crystals although in some cases X-ray diffraction data and etching experiments indicate 180° twinning. Single-crystal whiskers have also been obtained from sublimation of gallium phosphide crystals or powder and are also observed to electroluminesce. Undoped crystals are spectrographically pure except for traces of silicon. The effect of the silicon impurity on the luminescent properties is not yet known. (Some silicon-free samples have been prepared. Hence, it is not known whether silicon is actually present in the gallium phosphide lattice or is merely a surface contaminant.)

The electroluminescence emission spectra were obtained with a Bausch and Lomb grating monochromator and a 1*P*21 photomultiplier detector, the output of which was fed to a Moseley X–Y recorder. A potentiometer attached to the wave-length drive of the monochromator provided a linear wave-length scale on the X axis of the recorder. All spectra were corrected later on an equal energy basis for the spectral response of the detector. Silver paint electrodes were used for all the measurements reported here. They are rectifying with breakdown potentials of a few volts. In all cases the light emission originated at the negative electrode.

A typical electroluminescence emission spectrum of an undoped crystal is shown in Fig. 1. This resembles the spectrum reported by Grimmeiss and Koelmans except for the relative peak heights, which are markedly different. In their spectrum the height of the longer wave-length peak is roughly twice the height of the shorter wave-length peak. Their measurements, however, were made at low temperature, where internally excited photoluminescence could enhance the longer wave-length emission.

Emission spectra taken at room temperature and at about − 100° C. from a weakly zinc-doped crystal (approximately 0·001 per cent zinc) are shown in Fig. 2. The unique 'zinc' emission is clearly seen. Its peak at 7000 Å. is in contrast to the 5700 Å. and 6500 Å. peaks obtained from undoped crystals. It is more than an order of magnitude greater in energy than the 'undoped' emission which can be seen in Fig. 2 as a small peak near 6000 Å. The structure in the 'undoped' emission is masked by the overlap of the 7000 Å. band. The ordinates of the two spectra in Fig. 2 have been normalized to percentage of maximum brightness. The two spectra coincide,

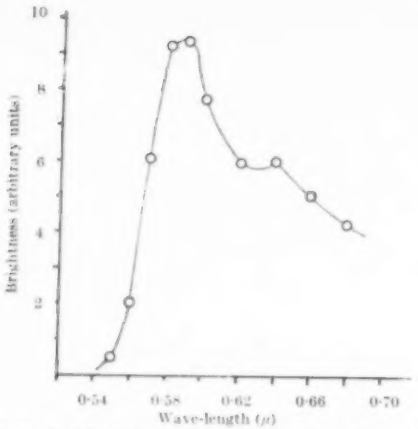

Fig. 1. Electroluminescence emission spectrum from undoped gallium phosphide crystal

approximately ; the deviations have not yet been determined to be significant.

The results corroborate the observation of Wolff, Hebert and Broder, that a unique red electroluminescence is obtained from zinc-doped gallium phosphide. I have found, however, that the zinc concentration is critical ; above about 0·01 per cent zinc only non-luminescent crystals are obtained. The lower limit on the zinc concentration is not known as yet. I am conducting experiments to correlate the amount of 'zinc' emission with the zinc concentration. It is possible that Grimmeiss and Koelmans did not observe the 7000 Å. emission because the concentration of zinc in their weakly doped crystals was too low, and in their heavily doped crystals too high.

The role of the zinc impurity in the luminescent process is not known. It is tempting to draw the analogy with copper in zinc sulphide. However, junction and impurity band formation have also been shown to play an important part in carrier injection electroluminescence[6], and it is conceivable that the zinc impurity is involved in this way.

I wish to acknowledge the assistance of Messrs. E. L. Peirson and C. V. Osterday in the preparation of the crystals, and am particularly grateful to Prof. J. J. Dropkin for reading and commenting on this manuscript.

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The Unit of Neutron Flux

We wish to support the suggestion[1] that a unit of neutron flux be defined. We feel, however, that it would be more practical to define the chad as follows :

$$1 \text{ chad} = 10^{12} \text{ n./cm.}^2 \text{ sec.}$$

The flux in most existing or contemplated power reactors lies in the range of 10^{12}–10^{13} n./cm.^{2} sec., the flux in zero energy reactors is generally about 10^9 n./cm.^{2} sec., and that encountered in sub-critical assemblies is approximately 10^6 n./cm.^{2} sec. High-flux research reactors operate at flux-levels ranging up to about 10^{16} n./cm.^{2} sec. The whole range of applied neutron physics could therefore be covered conveniently by the chad, as defined above, and the associated units microchad (10^6 n./cm.^{2} sec.), millichad (10^9 n./cm.^{2} sec.) and kilochad.

While having no objection in principle to the definition suggested earlier (1 chad = 1 n./cm.^{2} sec.), we feel that our definition is more practical and convenient in that, in addition to eliminating the clumsy compound nomenclature, it will remove the necessity for inclusion of large powers of 10.

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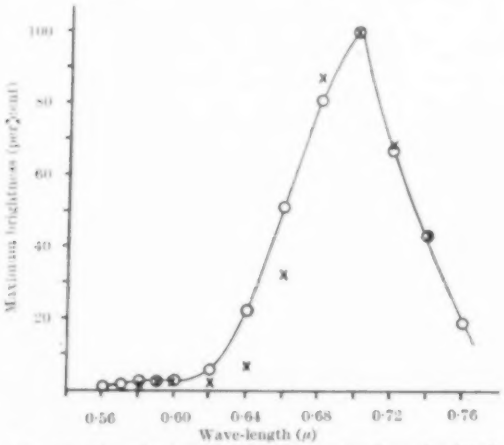

Fig. 2. Electroluminescence emission spectra from zinc-doped gallium phosphide crystal at room temperature and −100° C. (O, room temperature ; ×, −100° C.

CRYSTALLOGRAPHY

Some Electron Microscopic Observations on Single Crystals of Polyethylene

Some time ago it was found that in dilute solutions of polyethylene, particularly of 'Marlex 50', single crystals may be formed which can be made visible by means of electron microscopic techniques[1–3]. Morphologically, these crystals showed much resemblance to single crystals of paraffin, which were observed for the first time by Dawson and Vand[4] : the latter show a spiral terrace growth, the height of the terraces corresponding to the length of the chain molecules.

Keller[1] pointed out that the chains in the polyethylene crystal must necessarily be folded and that the height of the terraces corresponds to the distances between successive folds. He also noted that the crystals lose their crystallinity in the electron microscope, but that their morphological shape is probably preserved.

In an attempt to study undecomposed specimens, in which freshly prepared specimens were exposed

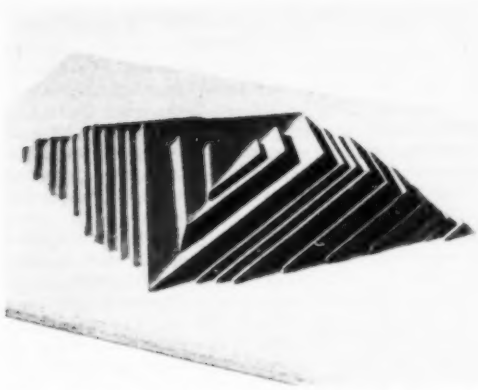

Fig. 1. Model of the striated structure of a polyethylene crystal

to a low-intensity electron beam, Keller and O'Connor[5,6] obtained completely different pictures. Although the crystal was still lozenge-shaped, it revealed a distinct division into four sectors, which were characterized by striations running non-parallel to the crystal boundaries, but which in our view were distinctly arranged in the (310) direction.

Some time ago we studied these types of crystals by electron microscopic stereo-techniques. From the micrographs we gained the impression that the crystals possess a pyramid-like structure. Using this technique, two micrographs are secured with the specimen set at different angles to the beam. As there was no visible difference between the two pictures, we concluded that the striations cannot possibly be due to extinction effects, as is sometimes stated in the literature.

Application of this technique requires that two pictures be taken of each crystal; owing to the change of the crystal in the electron beam, however, it is quite exceptional that two suitable micrographs will be obtained. To obviate this difficulty, we decided to use replica techniques. After some initial troubles had been overcome, we succeeded in securing pre-shadowed carbon replicas of polyethylene crystals.

We then took stereographs of these replicas; the first micrographs thus obtained completely confirmed our observation mentioned above with regard to the pyramidal structure. Some of the plates also revealed that the striations are straight. A model made of these structures is shown in Fig. 1. As can be seen from this model, the crystals do not have a true pyramidal structure, but can be better represented by a pyramid with a depressed apex. Mention ought to be made here of a statement by Bassett, Frank and Keller[7] that a pyramidal structure might be expected.

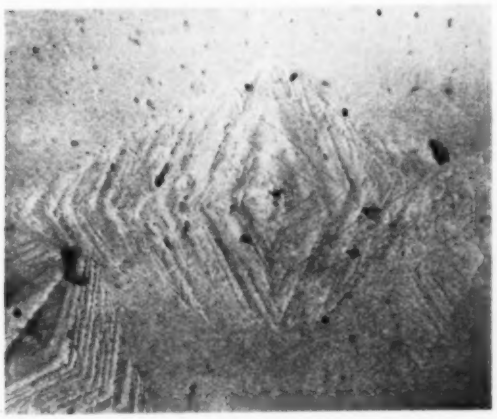

Fig. 2. Replica of a crystal of polyethylene. (× 15,000)

Although several attempts have been made in the past at explaining this striated structure, attention has never been directed to the relation between this structure and the spiral terraces observed in a later stage. In some of our replicas, both the pyramidal and the terraced structure can be seen (Fig. 2).

In view of the information provided by these micrographs, we expect that it will become possible to shed more light on the relation noted by us, and in general on the way in which polyethylene crystallizes from solution.

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CHEMISTRY

Localization of Ferrous Corrosion by Azide

Among the very large number of rust inhibitors, one of the simplest in structure, sodium azide, has received only scant attention. I have made some observations on the interaction between ferrous materials and neutral aqueous solutions of azide at ambient temperature.

Mild steel normally remains immune under azide solution. In sufficient concentration, this additive counteracts the corrosive effect even of chloride[1]. However, if mild steel is submerged in 1 *M* sodium azide containing 0·2 *M* sodium chloride, a scratch is made on the metal with a glass needle, and an anodic potential applied (for example, 2 V. vs. a stainless steel rod as cathode), a sharp red line appears instantly along the scratch, as if the steel were bleeding. This demonstration of highly localized attack is, however, only successful if no more than a few minutes elapse between the 'wounding' and the application of potential. Otherwise the scratch immunizes itself, obviously by growing an oxide film under the galvanic action of dissolved oxygen. If the scratch is applied in air, healing by dry oxidation is less rapid, and the lesion may be developed by submersion and anodic treatment after much longer intervals of time. The red ferric azide complex issuing from the scratch later diffuses away and hydrolyses, leaving a cloud of ferric hydrate.

A high-carbon steel, several low-alloy steels, a variety of cast irons, and 13 per cent chromium iron behaved quite similarly. A ferritic 17 per cent chromium iron also gave a 'bleeding' effect, but in this case the electrolysis had to be applied not later than a few seconds after the steel had been scratched

under the azide/chloride solution. Austenitic 18–8 type stainless steels gave no effect, even if they were made anodic while being scratched. The effect may therefore be developed as a sorting test for steels and perhaps also for showing corrodible areas in welds.

Voltage and concentrations are not critical for the effect to occur. Chloride may be replaced by bromide or iodide, but in the absence of halide the scratch does not develop. Attempts were made to replace azide by thiocyanate, which is iso-electronic with azide and also forms a red ferric complex. Thiocyanate, however, showed no localizing action. It is an anodic stimulator of corrosion, not an inhibitor.

The role of azide in these experiments is dual: as an inhibitor of the attack, and as a reagent of the product of attack. Similar effects can be produced with pairs of additives in which these two functions are separated; for example, with benzoate as inhibitor and ferroin as reagent. Also oxidation need not be electrolytic but can be galvanic, using oxidants such as persulphate. Ferricyanide can be used as oxidant and reagent in one, as in the 'ferroxyl test' for showing up pores in tin coatings of steel. Conspicuous localized corrosion should, then, be generally favoured by four factors: (*a*) oxidizing conditions, (*b*) the presence of a stimulator, (*c*) the presence of an inhibitor and (*d*) the presence of a reagent. Table 1 gives examples of such combinations by which corrosion limited to a scratch could be produced.

Table 1. Sets of Conditions leading to Scratch Attack of Mild Steel

Oxidant	mol./l.	Stimulator	mol./l.	Inhibitor	mol./l.	Reagent	mol./l.
2 Volts		Chloride	0·2	Sodium azide	1·0	Sodium azide	1·0
Ferricyanide	0·2	,,	0·0025	*Cyclo* hexylamine	0·05	Ferricyanide	0·2
2,4-Dinitroresorcinol	0·001	,,	0·001	*n*-Decoic acid*	0·001	2,4-Dinitroresorcinol	0·001
2 Volts		,,	0·001	*n*-Decoic acid*	0·01	*o*-Phenanthroline	0·005
Persulphate	0·02	,,	0·001	*n*-Decoic acid*	0·01	,,	0·005
2 Volts		,,	0·001	Sodium picrate	0·01	,,	0·005
Persulphate	0·02	,,	0·001	Sodium picrate	0·005	,,	0·005
2 Volts		,,	0·001	Maleic hydrazide*	0·05	,,	0·005
Persulphate	0·02	,,	0·001	Maleic hydrazide*	0·01	,,	0·005
Persulphate	0·02	,,	0·001	2-Methyl piperazine	0·02	,,	0·005

* Adjusted with sodium hydroxide to *p*H 7.

It is of interest to note that azide has none of the attributes normally held responsible for inhibition of rusting: in contrast to the structurally similar nitrite, and to chromate or picrate, it cannot act as an oxidant. It differs from the carboxylates in that it does not precipitate iron from solution, and from the amines by the absence of alkaline character. Unlike the proprietary rust preventives, it has a very simple structure. Further work on the azide/iron interaction may help to throw light on the still obscure mechanism of rust prevention by soluble additives.

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Hydrogen Bonding in Apatitic Calcium Phosphates

It is well known that the formula of naturally occurring and of precipitated synthetic hydroxyapatite is $Ca_{10}(PO_4)_6(OH)_2$: the molar Ca/P ratio of these compounds is 1·667. The compound mentioned can adsorb a consistent amount of water. For other synthetic calcium phosphates, with lower Ca/P ratios, the X-ray diffraction spectrum remains the same.

By combining data principally obtained from infra-red spectrometry, X-ray diffraction and chemical methods of investigation, we have established that the general formula of the apatitic calcium phosphate series is: $Ca_{10-x}(H)_x(PO_4)_6(OH)_{2-x}$, the value of x varying from 0 to 2. Detailed results concerning this problem have been presented in a preliminary report with some restrictions appropriate to such an over-simplified view[1]. According to our conception, some PO_4^{---} groups of these compounds are in fact $HPO_4^{=}$ groups which are linked by hydrogen bonds: $(O_3PO—H\cdots OPO_3)$. In our work, special attention has been paid to hydrated tricalcium phosphate (Ca/P ratio = 1·5), or $Ca_3(O_3PO—H\cdots OPO_3)(PO_4)_4(OH)$.

Others have reported similar findings in this field[2–4]: they point out that pyrophosphate, derived from these acidic groups by heating below 600° C., may be determined by chemical means and are also present in bone and teeth.

A paper published recently[5] gives us the opportunity to present some complementary information. According to these authors, infra-red spectra obtained from apatitic calcium phosphates with a Ca/P ratio between 1·667 and 1·5 show a band located at 3,400 cm.$^{-1}$ which is attributed to the O—H valence vibration of the $(O_3PO—H\cdots OPO_3)$ groups. The intensity of this absorption increases when the Ca/P ratio of the compounds considered decreases: apparently, this observation agrees with the conception of Posner *et al.*[5], the amount of $HPO_4^{=}$ groups being inversely proportional to the Ca/P ratio. However, it does not agree with the findings of other workers[6–9], who have shown that this vibration absorbs at about 2,850 and 2,400 cm.$^{-1}$. Moreover, when the frequency 3,400 cm.$^{-1}$ is plotted on the graph obtained by Lord and Morrifield[9], the distance O—O corresponds to 2·85 Å.; on the other hand, the distance observed for acidic phosphates is only 2·5 Å.[9,10], and this value agrees with the location of an absorption band at 2,400 cm.$^{-1}$. In our records, obtained with apatitic calcium phosphates with a Ca/P ratio lower than that of hydroxyapatite, we have actually observed a weak band located at 2,400 cm.$^{-1}$ and another one, with a high intensity, at 3,400 cm.$^{-1}$. The latter is also present on the spectrum of synthetic hydroxyapatite and consequently is not specific to the compounds with a Ca/P ratio lower than 1·667: as is well known, it corresponds to adsorbed water.

Probably, Posner *et al.*[5] have compared synthetic apatitic phosphates to natural hydroxyapatite, which does not contain adsorbed water and shows no band at 3,400 cm.$^{-1}$ as it has been verified by us. The inverse proportionality observed by them between the Ca/P ratio and the intensity of the 3,400 cm.$^{-1}$ wave may be explained as follows: the lower the Ca/P ratio of the synthetic calcium phosphates, the smaller are the crystals and the greater is the total surface of these crystals: in this way, the amount of adsorbed water is greater, as can be shown

experimentally. Like Posner *et al.*[5], we have observed on the infra-red spectra obtained with natural hydroxyapatite a band (3,560 cm.$^{-1}$) corresponding to the vibration of free OH groups. The same band appears when larger crystals of hydrated tricalcium phosphate, obtained by treating in the hydrothermal bomb, are studied: in this way, the amount of adsorbed water is much smaller than in the untreated compound, and the band corresponding to free OH groups clearly appears.

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Calcium Exchange of Bone Salts considered as a Measure of the Crystal Surface

Comparing the *in vitro* calcium-45 uptake by bone salts (ashed by a method slightly modified from that of Gabriel) from alcoholic and aqueous calcium-45 labelled calcium chloride solutions, we have been able to demonstrate the occurrence of remodelling phenomena (chemical reaction between the solid and the ions dispersed in the liquid phase, recrystallization *sensu stricto*) and of an isoionic exchange, which reaches an equilibrium when 8 per cent of the calcium ions of the solid are involved. All remodelling processes being discarded, we believe that this figure corresponds to a pure exchange value. In our conditions, the intracrystalline ion exchange by thermal vibration is too slow to interfere with the surface exchange process[1].

In a recent paper[2] Engström, correlating X-ray diffraction data and electron microscopy investigations, states that the needle-like crystal of bone salt has an average diameter of about 50 Å. This figure is also given by Molnar[3].

If the needle-like crystal is considered as a cylinder of the same length, the base surfaces not being taken into account, one may calculate, as a percentage, the ratio—plane volume of the surface/volume of the crystal—in the following way:

$$\frac{\pi h(2Rd - d^2)}{\pi R^2 h} \times 100 \text{ (per cent)}$$

where h is the length of the crystal; R is the radius of the crystal base; d is the third dimension of the surface plane volume. After simplifying, it becomes:

$$\frac{2Rd - d^2}{R^2} \times 100 \text{ (per cent)}$$

which in the particular case of bone crystals gives:

$$\frac{4,900}{625} = 7{\cdot}85 \text{ per cent}$$

when d is equal to the unit (1 Å.).

It is interesting to point out that the length of the crystal is of no importance for this calculation. This calculated figure corresponds closely to the experimental exchange value in such a way that the latter may be considered a real measure of the crystal surface. This assumption implies that the composition of the surface is not different from the composition of the bulk of the crystal. If the ratio is expressed in m.^{2}/gm. we obtain a value 250 m.^{2}/gm., which also fits the published figures[4].

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Lifetime of the Pyrene Dimer

The appearance of a new fluorescence emission band in concentrated solutions of pyrene was attributed by Förster and Kasper[1] to an excited dimer formed by combination of an excited singlet molecule with an unexcited molecule. Stevens and Hutton[2] have recently reported the lifetime of the dimer emission to be $1{\cdot}8 \times 10^{-3}$ sec. During tests of a new photoelectric spectrophosphorimeter an attempt was made to record this long-lived emission. The instrument was a modification of a spectrofluorimeter previously described[3], but in which two 800 c./s. choppers were used, one being situated in the beam of the exciting light and one in the beam of the fluorescence light. The choppers were driven by synchronous motors, and by rotation of one of the motors the choppers could be put into or out of phase so that the fluorescence monochromator could be made to record either the sum of the fluorescence and phosphorescence spectra or the phosphorescence spectrum alone. By adjustment of the photomultiplier voltage and by variation of the amplifier gain, the overall sensitivity could be increased by a factor of 10^4.

The operation of the instrument was checked by measurement of the visible phosphorescence from a 0·005 per cent solution of eosin in anhydrous glycerol which was reported by Mlle. Boudin[4] to have a lifetime of about 10^{-3} sec. We found the ratio of the intensities of fluorescence and phosphorescence to be, within a factor of two, the same as the value reported by Mlle. Boudin.

The efficiency of the choppers was tested by measurements on aerated solutions of fluorescein and anthracene, and it was found that the recorded

intensity of the fluorescence was reduced by a factor of more than 10^3 when the choppers were moved to the out-of-phase position.

A 10^{-2} *M* solution of Light's pure pyrene in B.D.H. spectroscopically pure cyclohexane was thoroughly de-aerated and sealed in a silica optical cell. The total luminescence spectrum was first measured using two different wave-lengths for excitation, namely, 366 mμ with viewing at right angles to the beam of exciting light, and 313 mμ with viewing in the same direction as the beam of exciting light. The spectrum was the same in each case and was similar to that reported by Forster and Kasper, and by Stevens and Hutton. The measurements were then repeated, but with the choppers out of phase and with the sensitivity increased by a factor of 10^4. The apparent phosphorescence emission from the dimer, measured in this way, had an intensity less than 10^{-5} times that of the total luminescence from the dimer. It was thus estimated that a component of the dimer emission having the lifetime reported by Stevens and Hutton must have an intensity less than 10^{-5} times the total dimer emission and that the greater part of the dimer emission must have a lifetime less than 5×10^{-5} sec.

The measurements with our spectrophosphorimeter set an upper limit to the lifetime of the pyrene dimer. A lower limit can be determined by measurement of the oxygen-quenching of its luminescence. Saturation of the 10^{-2} *M* solution with air was found to reduce the intensity of the monomer luminescence by a factor of about 7 and that of the dimer luminescence by a factor of approximately 19. In a 10^{-3} *M* solution, where dimer formation can be neglected, saturation with air reduced the intensity of the monomer luminescence by a factor of about 22. Assuming that the dimer is formed by combination of an excited singlet molecule with a normal molecule, and that oxygen quenching of both the monomer and dimer is collisional, it can be shown that :

$$F_D^0 / F_D = F_M^0 / F_M (1 - k_d \tau_d [O_2]) \quad (1)$$

where F_D is the intensity of dimer luminescence in 10^{-2} *M* solution containing oxygen, F_D^0 is the corresponding value in the absence of oxygen, F_M and F_M^0 are the corresponding intensities of monomer luminescence in the same solution, k_d is the bimolecular oxygen quenching constant for the dimer and τ_d the lifetime of the dimer in the absence of oxygen. The encounter frequency in *cyclo*hexane at 20° C. (that is, the maximum possible value for k_d) is $6{\cdot}8 \times 10^9$ litre mole^{-1} sec.$^{-1}$, and the oxygen concentration in an air-saturated solution is $2{\cdot}5 \times 10^{-3}$ *M* (ref. 5). Hence $\tau_d > 10^{-7}$ sec. The corresponding values for the monomer lifetime are $\tau_M > 3{\cdot}5 \times 10^{-7}$ sec. in 10^{-2} *M* solution and $\tau_M > 1{\cdot}2 \times 10^{-6}$ sec. in 10^{-3} *M* solution. The latter value is in approximate agreement with the value of $0{\cdot}5$–$1{\cdot}0 \times 10^{-6}$ sec. which we estimated from the intensity of the long wave-length absorption band system.

The evidence from our experiments gives a value for the lifetime of the dimer which is at least thirty times less than that found by Stevens and Hutton, a discrepancy for which it is difficult to find an explanation. One possibility which we considered was that the long-lived emission was due to solute or photochemical decomposition products, adsorbed on the wall of the vessel (it being assumed that any luminescence from the vessel wall would have been included in the total phosphorescence spectrum measured by Stevens and Hutton). However, no evidence of a 'wall-adsorption' effect was found in our experiments with 10^{-3} *M* pyrene solution in the silica cell, the ratio of 'phosphorescence' to fluorescence being the same, within experimental error, whether right-angle illumination (366 mμ) or frontal illumination (313 mμ) was used (in the latter arrangement the illuminated cell face, and hence also the luminescence from any adsorbed matter, was viewed by the photomultiplier). Whatever the cause of the discrepancy it is sufficiently important to merit further investigation.

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We previously reported[1] a value of $1{\cdot}8 \times 10^{-2}$ sec. for the lifetime of the pyrene dimer in de-aerated *cyclo*hexane solutions. This value was obtained from the exponential decay of the emission observed from a flashed solution, the order of magnitude being confirmed by photographing the emission excited by a constant intensity source through a Becquerel phosphoroscope in which the time interval between excitation and observation was $\sim 5 \times 10^{-4}$ sec. Although it was not actually stated, it was implied that this lifetime referred to the total dimer emission.

In an attempt to repeat this observation Parker and Hatchard (preceding communication) report that they were unable to record an emission component of this duration for a similar solution and conclude that if such a component is present it must have an intensity less than one part in 10^5 of the total dimer emission which has the much shorter lifetime of 5×10^{-5}–10^{-7} sec.

We have again measured the lifetime of the long-lived component by recording the pyrene emission from the phosphoroscope on a Mazda 27*M*3 photomultiplier tube and photographing its signal on a Solartron oscilloscope type *CD* 7118.2 ; the apparatus was tested by measuring the emission lifetime of uranyl sulphate crystals for which the observed value of $2{\cdot}53 \pm 0{\cdot}03 \times 10^{-4}$ sec. is in excellent agreement with the published[2] lifetime of $2{\cdot}55 \times 10^{-4}$ sec. For a 10^{-2} *M* solution of pyrene a component of lifetime $1{\cdot}75 \pm 0{\cdot}1 \times 10^{-2}$ sec. was observed which confirms the value previously obtained.

In order to measure the ratio of slow to fast components of the dimer emission, the apparatus was modified as described by Williams[3] and the intensity of the slow component was found to be 0·2 per cent of the total dimer emission. Clearly our previous implication that the two different emission spectra observed from pyrene solutions are respectively associated with slow and fast emission components was unjustified and the system of electronic states must be more complex than we at first visualized.

At lower temperatures (— 10° C.) in liquid paraffin we have observed a value of 60×10^{-3} sec. for the long-lived pyrene component ; this could be ascribed to the suppression of an energy-dependent first-order radiationless decay of the dimer which should lead

to overall self-quenching of fluorescence in pyrene solutions at room temperature. It is possible, however, that the residual oxygen in the outgassed solutions partially quenches the long-lived component at room temperature but is much less effective in the more viscous paraffin at − 10° C. The radiative lifetime of the slow component may therefore exceed 60×10^{-3} sec. and in the complete absence of oxygen this component may contribute appreciably to the total intensity of dimer emission.

The problems associated with the outgassing of solutions are not simplified by the observation that the pressure in a vessel sealed-off at 10^{-7} mm. may be increased to as much as 10^{-2} mm. by the act of sealing-off (Heavens, O. S., private communication): since quartz requires a higher seal-off temperature it is possible that the failure of Parker and Hatchard to observe the slow component is due to a comparatively high pressure of gas released from their quartz vessels during seal-off.

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BIOCHEMISTRY

Origin of Urinary Pyrocatechol

The formation of pyrocatechol in rats fed shikimic and quinic acids has recently been reported by Booth *et al.*[1]. They proposed that human urinary pyrocatechol might have a similar origin since these acids are present in some edible plants. However, pyrocatechol also could arise from the oxidation of phenol, a compound formed in the gut by the action of bacteria on protein[2]. If pyrocatechol excretion-rates were shown to be dependent on gut bacteria, support for the proposed relationship would be obtained. An examination was therefore made of the pyrocatechol excretion-rates in several normal persons who later were treated with large doses of neomycin, a drug which can virtually sterilize the gut.

Pyrocatechol was determined fluorometrically by an adaptation of the method of Weil-Malherbe and Bone[3]. An aliquot of the urine, adjusted to *p*H 1 with hydrochloric acid, was heated to 100° C. for 10 min. and then made neutral by the addition of dilute base. An alumina adsorbate was prepared, and an aliquot allowed to react with the ethylenediamine reagent. The neutral fluorescent derivative which formed was extracted into *iso*butanol and its fluorescence compared with that of pyrocatechol using a Farrand photofluorometer. The specificity of the method for urinary pyrocatechol was determined by paper chromatography of the concentrated *iso*-butanol extract. Under ultra-violet illumination only one major fluorescent spot was seen which had the same R_F and blue-green colour as similarly prepared pyrocatechol or norepinephrine. It has been shown that identical ethylenediamine derivatives are formed from both pyrocatechol and norepinephrine[4]. Since only trace amounts exist in urine no correction need be made for the norepinephrine.

While pyrocatechol excretion-rates in the normal person exhibit a large diurnal variation, daily excretion-rates remain rather constant[5]. When neomycin is ingested in large doses, however, the daily excretion-rates of pyrocatechol decline rapidly as shown in a typical result listed in Table 1. From these results a major fraction of urinary pyrocatechol appears to be dependent upon the activity of gut bacteria. Since phenol can be converted to pyrocatechol by the animal organism[6], the formation of the major urinary catechol may proceed as follows:

tyrosine ——→ phenol ——→ pyrocatechol

Although the conversion of tyrosine to phenol appears to be mediated by an enzyme present in coliform bacilli[7], further oxidation may be non-enzymatic. The site of this oxidation could be within the gut since pyrocatechol has been reported present in faeces[5], but oxidation of unconjugated phenol by other body organs probably can occur.

Table 1. Pyrocatechol Excretion-Rates before and during the Administration of Neomycin in a Daily Oral Dose of 8 gm.

Pre-treatment period		During neomycin treatment	
Days	Pyrocatechol excretion (mgm./day)	Days	Pyrocatechol excretion (mgm./day)
1	6·0	4	3·2
2	5·2	5	1·2
3	5·8	6	1·6

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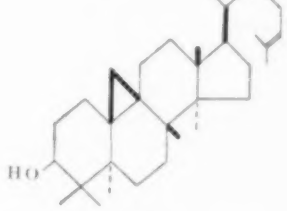
Cyclo-Arthenol: a Minor Constituent of Linseed Oil

Cyclo-Arthenol has been isolated by Barton[1] in addition to the corresponding ketone, *cyclo*-arthenone, from the latex of the *Artocarpus integrifolia* fruit and afterwards by Spring and co-workers from the unsaponifiable part of *Strychnos nux vomica*[2,3]. These authors have studied its structure and have demonstrated that *cyclo*-arthenol may be correctly described as 9 : 19-*cyclo*lanost-24-en-3β-ole:



Other compounds of similar nature are *cyclo*-laudenol[4], *cyclo*-eucalenol[5] and 24-methylene*cyclo*-artanol[6], all of which are characterized by the presence of a *cyclo*-propane ring at 9–19.

In the course of work on the constituents of the unsaponifiable fraction of vegetable oils I have

Table 1

	Alcohol $C_{30}H_{50}O$, from linseed oil		Data quoted by:									
			Barton (ref. 1)		Spring (refs. 2, 3)		Gonzales (ref. 10)		Chapon (ref. 11)		Shimizu (ref. 6)	
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$
Alcohol	115–116	+51·4	85–92	+48	115	+54	97–112	+47	80–105	+48·5	114–115	+52
Acetate	121–122	+60·2	122·5	+58	122–124		118·2	+54	119–120	+58	122–124	+59
			123·5			+59·5						
Benzoate	129–130	+68	129–130	+65	130	+76	129	+54	130	+67	129–130	+75

isolated, by chromatography of the neutral[7] unsaponifiable of a linseed oil of Italian production on silicic acid, a group of materials characterized by a yellow-brown Liebermann–Burchard reaction. Paper chromatography[8] shows the presence in this fraction of three different substances at least, probably terpenic alcohols. Subsequent chromatography of the fraction on aluminium oxide made it possible to isolate, by elution with hexane–benzol, a substance which after crystallization from methanol melted at 115–116° C., $[\alpha]_D = +51{\cdot}4°$. This substance gave a yellow-brown colour with the Liebermann reagent and a distinct yellow colour with nitromethane; paper chromatography showed the presence of only one component.

The compound isolated has been characterized through the acetate and the benzoate; in Table 1 are recorded the values of specific rotations and of the melting points, which agree satisfactorily with those obtained for the *cyclo*-arthenol by the authors mentioned.

Identification has been confirmed through the infra-red spectrum of the compound isolated from linseed oil, which was identical with that reported by Cole[9] for the *cyclo*-arthenol. Finally, the mixed melting point of the acetate of the compound, with a sample of genuine acetyl-*cyclo*-arthenol, kindly supplied by Dr. Spring, did not show any decrease of melting point.

This investigation suggests that triterpenic alcohols of the same group may be present in other animal and vegetable oils, such as olive oil, maize germ oil, peanut oil and tallow oil.

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A Bilatriene Pigment in the Green Terebellid Polychæte *Eupolymnia heterobranchia* (Johnson)

Eupolymnia heterobranchia (Johnson) is a relatively large terebellid found in mud and under boulders on the Pacific coast of North America. All the specimens that I have found near Friday Harbor, Washington, had a deep green colour. This is due to granules of green pigment deposited in the coelomic cells and in the epidermis. The pigment was readily extracted with acidified methanol, gave a good Gmelin reaction, and reacted positively to the zinc bilipurpurin test, demonstrating that it was a bilatriene. The red-fluorescent compound obtained in the latter test had a sharp maximum at 625 mμ (Unicam), indicating the presence of meso- rather than proto- side chains[1]. The great resistance of the pigment to concentrated sulphuric acid also distinguished it from biliverdin. In a 5 per cent hydrochloric acid : methanol mixture the absorption spectrum showed peaks at 360 and 675 mμ, resembling mesobiliverdin hydrochloride (363 and 670 mμ; ref. 1).

Mesobiliverdin has been found to occur in only a few animals, and these are all phytophagous insects[2]. The occurrence of a similar pigment in *Eupolymnia* is interesting in view of the report by Berkeley and Berkeley[3] that orange-red individuals are sometimes found. It would be most interesting to know whether the appearance of these orange worms is due merely to a lack of the green pigment or, as is just possible, to mesobilirubin or related pigment.

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Occurrence of *Cyclo*-Propene Acids in Some Plants of the Order Malvales

Sterculic and malvalic acids are the only fatty acids known to contain a *cyclo*-propene ring in their structures. From the seed oil of *Sterculia foetida* (Sterculiaceae), Nunn[1] isolated sterculic acid (C_{19}) and defined its structure. Malvalic acid (C_{18}) isolated from Malvaceae by Shenstone and Vickery[2] has been structurally identified by Macfarlane, Shenstone and Vickery[3] and Craven and Jeffrey[4]. Afterwards we reported[5] the occurrence of minor amounts of sterculic acid associated with malvalic acid in the oils from some malvaceous plants. The occurrence of both acids in the seeds of *Sterculia foetida* and two species of Malvaceae has also been observed by C. R. Smith, jun., T. L. Wilson and K. L. Mikolajczak (private communication).

We have measured the concentrations of malvalic and sterculic acids in the seed and leaf oils of three species from each of the families, Malvaceae and Sterculiaceae. The solvent-extracted oils were saponified under mild conditions, and the total fatty acids were subjected to low-temperature crystallization. The filtrate contained almost all the *cyclo*-propene acids, and saturated acids were absent. Malvalic and sterculic acids were cleanly separated by the method of reversed-phase column chromatography[6]. They were closely preceded by, and sometimes merged with, linoleic and oleic acids, respectively.

The Halphen colour test, given by both *cyclo*-propene acids[3] but not by other unsaturated acids, was used to estimate the concentrations of malvalic and sterculic acids in the isolated chromatograph 'peaks'. The colour reaction given by each fraction was adjusted in a series of increasing dilutions to a point near visual extinction. The colours were then compared with a similar series obtained from standard concentrations of pure acids. The ratio of dilutions at similar colour intensities gave the concentration of *cyclo*-propene acid present in each impure fraction.

In the seed-oil of *Sterculia foetida* the colorimetric method was checked by an estimation of the products of partial hydrogenation of the fatty acids. In the hydrogenation, ethanol was used as a solvent with 5 per cent palladium on calcium carbonate as a catalyst. The *cyclo*-propane acids, dihydromalvalic and dihydrosterculic acids, together with small amounts of their methyl-branched derivatives, were separated from the straight-chain saturated acids by crystallization and afterwards isolated after reversed-phase and gas-liquid chromatography. The concentrations of the *cyclo*-propene acids estimated by the colorimetric and hydrogenation methods agreed within ± 2 per cent.

Malvalic and sterculic acids in the chromatographic fractions were identified after direct isolation by means of fractional crystallization. In some samples, however, identification was effected more readily by conversion of the *cyclo*-propene acids to the stable dihydromalvalic and dihydrosterculic acids.

Table 1. Concentrations of Malvalic and Sterculic Acids in Plant Oils*

Plant family and species	Source of oil	Malvalic acid (per cent)	Sterculic acid (per cent)
Malvaceae			
Malva parviflora†	leaves	6·9–13·9	3·1–6·1
Malva verticillata‡	leaves	1·5–3·7	0·3–0·8
Gossypium hirsutum	seeds	0·7–1·5	0·3–0·5
Sterculiaceae			
Brachychiton acerifolium	leaves	nil	nil
	seeds	6·1–8·1	1·4–1·9
Brachychiton populneum	leaves	nil	nil
	seeds	8·9	1·1
Sterculia foetida	seeds	4·4	45·6

* When expressed as percentages of the total fatty acids, these values will be increased by factors of 1·14–1·25 for the seed oils and 1·49–1·82 for the leaf oils.
† Total *cyclo*-propene acids in seed oil = 5·0 per cent.
‡ Total *cyclo*-propene acids in seed oil = 1·2 per cent.

The concentrations of malvalic and sterculic acids in the oils from several plant species are given in Table 1. In the oils containing *cyclo*-propene acids both occur together, and the concentration of malvalic acid exceeds that of sterculic acid in five of the six species examined. The seed oil of *Sterculia foetida* is exceptional in its high concentration of *cyclo*-propene acids and in the preponderance of sterculic acid. In oils where malvalic acid predominates, the highest concentrations of *cyclo*-propene acids occur in *Malva parviflora* (up to 20 per cent of the oil or 35 per cent of the total acids) and in *Hibiscus syriacus* (25 per cent of the total acids). The concentrations in freshly extracted, unrefined oils of cotton seeds (*Gossypium hirsutum*) were always low but in most samples fairly constant, while in *Malva* spp. the concentrations in the leaf oils were very variable. There is evidence from the chromatograms of the presence of other *cyclo*-propene acids, but so far it has been impossible to isolate sufficient amounts for identification.

We are indebted to Mr. K. E. Murray for isolation of the *cyclo*-propane acids by gas-liquid chromatography.

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Identification of Œstradiol-17β Testosterone and its Precursors from *Scylliorhinus stellaris* Testes

Since its first isolation by Laqueur *et al.*[1] in 1935 from tissue of steer testis, testosterone has been obtained from some species of mammals only, namely, horse and man, by Ruzicka *et al.*[2,3]. Furthermore, Brady[4], by *in vitro* experiments on the biosynthesis of testosterone from ^{14}C-acetate, indicated that testosterone is probably the androgenic testicular hormone in two additional species, rabbit and hog. Androgenic substances of non-mammalian vertebrates are still unknown. On the other hand, œstradiol-17β has been found to be the œstrogenic hormone not only in mammals but also in the elasmobranch *Squalus suckleyi*[5], in the lungfish *Protopterus annectens*[6], in birds[7] and in the starfish *Pisaster ochraceus*[8]. The investigation of the androgenic material in non-mammalian vertebrates seems therefore necessary to fill this gap, and is of obvious evolutionary importance.

We have started an investigation of the androgenic steroids by their direct determination in the testicular tissue of the elasmobranch *Scylliorhinus stellaris*. 110 gm. of material were obtained from six sexually mature animals and extracted according to the method of Ruzicka *et al.*[9]. The acetonic residue was refluxed with chloroform in order to eliminate urea, which is present in large amounts in elasmobranch tissue. The steroid fraction was separated by counter-current distribution. The neutral fraction (25 mgm.) was then resolved by paper chromatography in systems developed by Bush[10]. The phenolic fraction (81 mgm.) was chromatographed according to the system developed by Heftmann[11].

Neutral fraction. First chromatography in system 'Bush *C*' showed the presence at the starting line of blue tetrazolium-positive material, which probably corresponds to corticosteroids. Five spots were identified in successive paper chromatographies by short-wave ultra-violet light and rechromatographed. Three of these spots corresponded to testosterone, androstenedione and progesterone on the basis of the constant R_F values and absorption maxima. The spots corresponding to androstenedione and progesterone showed the characteristic colour blue-violet and blue, respectively, with the Zimmermann reaction. The spot corresponding to testosterone, after elution and oxidation with CrO_3, was rechromatographed in system Bush *A*. The oxidation product showed the same R_F as androstenedione.

According to semiquantitative estimation these steroids were present in the amounts of approximately

50 μgm./kgm. of testosterone, 70 μgm./kgm. of androstenedione and 100 μgm. kgm. of progesterone.

The two additional unidentified spots both showed absorption maxima at 240 mμ and the positive Zimmermann reaction. One of these gave an R_F value of 0·50 in system Bush B_3 (25 ± 1° C.); the other one gave an R_F value of 0·79 in system Bush A (25 ± 1° C.).

Phenolic fraction. The phenolic fraction resolved by paper chromatography and the ferric chloride plus $K_3[Fe(CN)_6]$ colour reaction showed the presence of oestradiol-17β amounting to about 20 μgm./kgm.

The present investigation has demonstrated that the androgenic substances secreted by the testis of the elasmobranch *Scyliorhinus stellaris* are the same as those of mammals. Furthermore, as in mammals, oestradiol-17β is present as well[2,12,13].

Especially interesting is the identification of progesterone, which has not been isolated before from testis tissue, but has been considered as a probable precursor of testosterone. In fact Shaunwhite and Samuels[14] demonstrated, by *in vitro* work, the biosynthesis of 17α-hydroxyprogesterone, androstenedione and testosterone from isotopically labelled progesterone.

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Occurrence of Catecholamines in Acrania and Invertebrates

The occurrence of catecholamines in vertebrates has been extensively studied in recent years after the development of simple and accurate methods for the quantitative assay of such compounds. Relatively few investigations have been carried out with regard to their presence in invertebrates, however.

Biedl[1] and Gaskell[2] concluded from biological assay of extracts of leeches and the earthworm that these contained adrenaline. Later Erspamer and Boretti[3] showed the presence of *p*-hydroxyphenylethanolamine (octopamine) in the posterior salivary glands of *Octopus vulgaris*. These glands were also found to contain noradrenaline[4].

In an extensive investigation Östlund[5] found 0·13 μgm. of adrenaline and 0·48 μgm. of noradrenaline/gm. in the ventral ganglionic chain of *Lumbricus terrestris*, thus confirming the presence of catecholamines in this annelid. Similar amounts were found in *Arenicola marina* (lugworm). In a variety of other invertebrates Östlund[5] found only traces of catecholamines, however (less than 0·01 μgm./gm.). In insects, on the other hand, he observed relatively large amounts of adrenaline, noradrenaline and dopamine.

In the present experiments various invertebrates were collected fresh and extracted with 3–5 volumes of 10 per cent trichloroacetic acid. The catecholamines were adsorbed on an alumina column and eluted with acetic acid. Adrenaline and noradrenaline were estimated by a fluorimetric technique according to Euler and Lishajko[6].

The results are presented in Table 1. It will be seen that adrenaline and noradrenaline could not be demonstrated with certainty in invertebrates except in the annelid *Lumbricus terrestris* and in the larvæ of *Pieris brassicae*, the common cabbage white butterfly. This is in agreement with the previous findings of Östlund[5], who found in larvæ from various insects from 0·01 μgm. to 0·075 μgm. of adrenaline and from 0·1 to 1·3 μgm. noradrenaline per gm. In the present work the adrenaline content in the *Pieris* larvæ was negligible, while the noradrenaline content was 0·33 μgm./gm. From these results it is obvious that noradrenaline not only occurs in remarkably high amounts in insects' larvæ but also that it occurs greatly in excess of adrenaline.

Table 1. Adrenaline and Noradrenaline in Invertebrates and Acrania (Whole Animals, in Mesothuria Visceral Organs)

Phylum	Species	Adrenaline (μgm./gm.)	Noradrenaline (μgm./gm.)	Total weight (gm.)
Aschelminthes	*Priapulus caudatus*	< 0·005	< 0·005	15
Mollusca	*Buccinum undatum*	< 0·005	< 0·005	23
	Mytilus edulis	< 0·005	< 0·005	250
Annelida	*Lumbricus terrestris*	0·003	0·015	80
	Eunenia crassa	< 0·005	< 0·005	125
Crustacea	*Carcinus maenas*	< 0·005	< 0·005	11
Insecta	*Pieris brassicae* (larvæ)	< 0·005	0·33	47
Echinodermata	*Mesothuria intestinalis*	< 0·005	< 0·005	20
Urochordata	*Ciona intestinalis*	< 0·003	< 0·005	30
Acrania	*Branchiostoma lanceolatus**	< 0·005	0·16	5·6

* Syn. *Amphioxus*.

The presence of definite quantities of noradrenaline in *Branchiostoma*, 0·16 μgm./gm. in the whole animal, is of some interest since it indicates that this primitive organism is capable of synthesizing noradrenaline. On the other hand, there was no evidence of adrenaline.

The results presented have thus confirmed, by fluorimetric technique, the presence of considerable amounts of noradrenaline in *Lumbricus terrestris*, larvæ of *Pieris* and in *Branchiostoma lanceolatus*. Adrenaline was also found, as previously noted[5], in small amounts in *Lumbricus*. In the other invertebrates studied, the amounts of adrenaline and noradrenaline, if present at all, were extremely small.

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Effects of Speciation on Serum Proteins in the Genus *Macaca* with Special Reference to the Polymorphic State of Transferrin

The effects of speciation on the serum proteins of macaque monkeys and other primates are being studied in our laboratory by a combination of starch-gel electrophoretic and immunological techniques[1,2]. In an initial survey of the sera from 19 cynomolgus monkeys (*Macaca irus*) and 17 rhesus monkeys (*Macaca mulatta*) we found that the cynomolgus sera contained a larger proportion of γ-globulin than the rhesus sera and also that all the cynomolgus sera showed only one transferrin whereas 10 of the 17 rhesus sera showed more than one transferrin. At the time, we observed that one of the ten rhesus sera showed three transferrin components and that in the other nine sera, each of which showed two components, the relative positions of the transferrins in starch-gel varied in some cases between the samples. In order further to define the polymorphic state of transferrin in the species *Macaca mulatta* and the apparent absence of this state in the species *Macaca irus*, the sera from 40 more rhesus monkeys, newly arrived from India, and from 40 more cynomolgus monkeys, newly arrived from the Philippines, were kindly provided for study by Parke, Davis and Co. The rhesus monkeys in this shipment were captured in an area the size of a township near the Nepal border. It was not possible to trace exactly where in the Philippines the cynomolgus monkeys were captured. We have now detected fourteen transferrin patterns in the rhesus monkeys and still only one pattern in the cynomolgus monkeys. Thirteen of the transferrin patterns were represented in the last shipment. Our work, then, not only independently confirms but also considerably extends the report by Blumberg[3] of eight transferrin patterns in rhesus monkeys. Our results are particularly interesting in indicating that while the environment of one species selects for a polymorphic state, the environment of a closely related species selects against this state.

Although two-dimensional starch-gel electrophoresis[4] in a discontinuous buffer system[5,6] followed by immunological examination of the separated, fast migrating β proteins clearly demonstrated whether one, two or three transferrin components were present in a serum sample, comparative one-dimensional starch-gel electrophoresis revealed the subtler differences in the mobilities of the transferrins. Fig. 1*A* presents a typical result gathered by this latter type of screening in which a number of sera are run side by side in a large gel. Note the uniformity of the transferrin band in the 5 cynomolgus sera in contrast to the variability of the transferrin bands in the five rhesus sera. The fourteen transferrin patterns, of which type I is identical with the cynomolgus transferrin band, are shown in Fig. 1*B*. The actual homology of the cynomolgus and rhesus transferrins to human transferrin was established by two-dimensional starch-gel immunoelectrophoresis[6]. Fig. 2 presents the results of an immunoelectrophoretic experiment in which we employed one of our unispecific, chicken antisera[7] to human transferrin.

The transferrin types represented in Fig. 1 can be subdivided into those showing one component, types I, II, III and IV; type V showing two components which almost merge; those showing two closely adjacent components, types VI, VII and VIII; those showing two components increasingly separated from each other, types IX and X, then types XI and XII, and finally type XIII; and the last showing three components, type XIV. Fig. 1 also lists the distribution of sera among these types. In this connexion the breakdown of sera among types I, II and V is still being checked, for a number of repeated determinations are needed to differentiate a large single band of transferrin from two which touch each other.

The genetics of the rhesus transferrins has still to be investigated. Yet by analogy to the human transferrins[8] we postulate that certain of these types (particularly those in the first sub-division) are con-

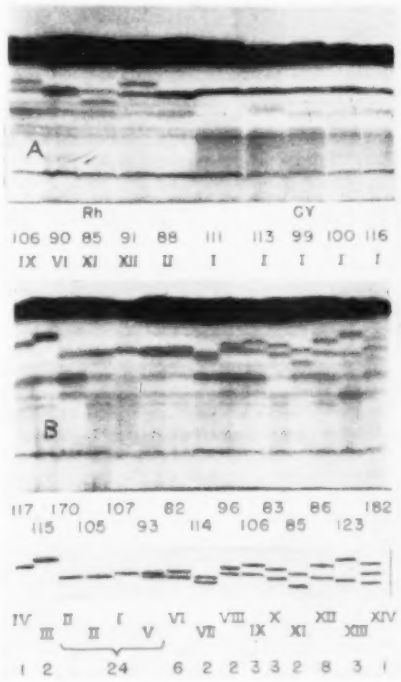

Fig. 1. *A*, Comparative one-dimensional starch-gel electrophoresis of five rhesus and five cynomolgus sera. The sample designations and the transferrin types of these samples are printed below the photograph of the gel. *B*, Comparative one-dimensional starch-gel electrophoresis of 15 rhesus sera representing the 14 transferrin types. Below the photograph of the gel and the sample designations, that portion of the photograph showing just the transferrin bands is presented diagrammatically. The numerical breakdown of the rhesus sera among the various transferrin types is presented below

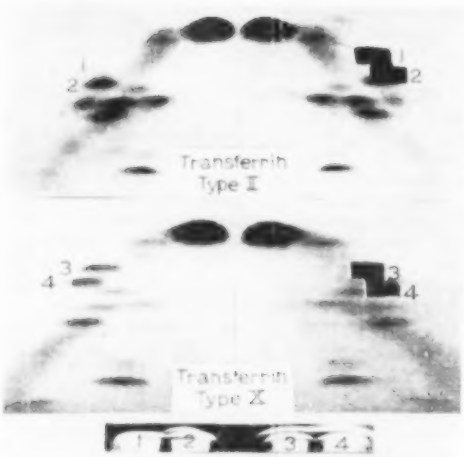

Fig. 2. Two-dimensional starch-gel immunoelectrophoretic identification of transferrin in type II and type X rhesus sera. One-half of each gel was first stained with amido black to show the distribution of protein components (photographs on the left). Then from the unstained halves pieces of gel, estimated to contain the transferrin or to serve as a control, are cut out. Then these halves are stained (photographs on the right). The pieces of gel are placed at the bottom of an immunoelectrophoretic chamber, overlaid first with agar, then with unispecific antiserum to human transferrin. After 12 days incubation the reactions were photographed (photograph on the bottom). Positive precipitin lines, demonstrating the presence of transferrin, were afforded by gel pieces 2, 3 and 4 but not by 1, the control piece. The lateral non-specific band which always forms just above the starch-gel may be discounted

trolled by homozygous combinations of genes and the other types by heterozygous combinations of genes. Although it would be necessary to test further specimens and other species of macaques to relate the frequencies of transferrins to the taxonomy of the genus, nevertheless it is clear that there are sharp species differences in the degree of polymorphism of transferrin.

In another approach to the detection of species differences in the state of the proteins, we found that our unispecific immune reagents could be used to measure as well as identify protein components in the macaque sera. For example, the relative γ-globulin concentration of each cynomolgus and rhesus sera was determined by immunochemical assay with a uni-specific anti-human γ-globulin reagent[8]. By this convenient means we clearly show that we are measuring the monkey protein equivalent to human γ-globulin. Though there is some overlapping of high rhesus and low cynomolgus values, the cynomolgus sera have a much larger mean γ-globulin concentration than the rhesus sera, 17·2 per cent as compared to 10·7 per cent. The difference in the means by the t test is significant at the 0·1 per cent level of confidence.

A more detailed account of the application of our experimental procedures to the study of primate proteins will be prepared later. It should be emphasized at this point, however, that the equivalent proteins in man and monkey are antigenically related, but not identical.

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PHYSIOLOGY

Polypnœa evoked by heating the Scrotum of the Ram

Thermal or electrical stimulation of discrete regions in the brain of several species can cause specific and vigorous thermo-regulatory responses[1–3]. It is equally clear that in sheep and calves, the polypnœa in response to raised environmental temperatures may start before the temperature of the blood supply to the brain changes[4,5]. It was concluded that in these ruminants the thermal stimulus to panting can be entirely peripheral in origin. Whether the receptors concerned were concentrated in special areas of skin or were uniformly distributed over the surface of the body remained unresolved.

During experiments designed to examine the thermo-regulatory properties of the Merino ram's scrotum, a reflex polypnœa was observed to be initiated and sustained when this region was specifically heated. In 12 experiments on 5 rams, this was examined by enclosing the closely clipped scrotum in an insulated chamber through which dried air was circulated continuously. Temperatures were recorded at least once every minute from thermo-couples in the air outflow from the chamber, in the rectum at a depth of 10 cm., and in some experiments on the mid-posterior surface of the scrotum. The air of the room was controlled at $17 \pm 2°$ C. throughout all experiments. Records were obtained for 1 hr. with the ram standing quietly and with air at $23 \pm 4°$ C. passing over the scrotum. Scrotal air temperature was then rapidly raised to either $45 \pm 1°$ C. (in 9 experiments) or $40 \pm 1°$ C. (in 3) for 1–1½ hr. A typical response is shown in Fig. 1.

The mean respiratory-rates during the control periods ranged from 34 to 66/min.; in most experiments the respirations were quiet and almost imperceptible. The respirations became more definite and their frequency increased while scrotal air temperature was rising. The mean respiratory-rates varied from 61 to 171/min. in the first 30 min. of heating, they increased to 114–185/min. during the next 30 min. and were 117–163/min. in the final 30 min. Maximum respiratory rates of more than 200/min. were recorded during 3 experiments involving 3 of the rams. The typical response was not open-mouthed

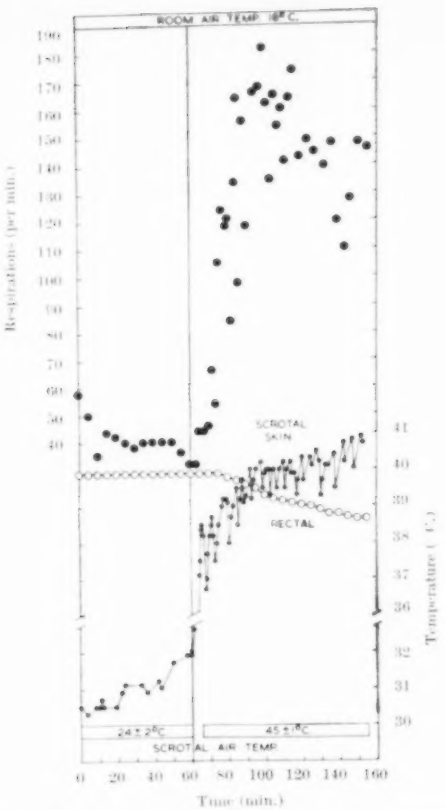

Fig. 1. The changes of respiratory-rate (●), rectal temperature (○–○) and scrotal skin temperature (●–●) induced by heating the scrotum of a ram with air at 45° C.

The falls of skin temperature occurring during the heating coincide with mass discharges from the sweat glands of the scrotum (Waites, unpublished observations)

panting, but a rapid polypnœa associated with a moist muzzle.

To confirm that the scrotal skin of each ram was specially sensitive to temperature, an area of mid-side skin equal to the area of the scrotum was close-clipped and heated with air at 45 ± 1° C. The respiratory response was negligible: mean frequencies were 45–65/min. during the control period, and 43–71/min. during the heating. It must therefore be concluded that the skin of the scrotum of the ram is particularly well endowed with peripheral thermal receptors.

No change in rectal temperature was detected before the start of the polypnœa, but thereafter rectal temperatures fell by 0·3–1·25 deg. C. in all experiments. In 7 of the 12 experiments, rectal temperature dropped at the rate of 0·5–1·0 deg. C./hr. and in these the fall started sharply within 8–18 min. after the onset of the polypnœa (Fig. 1). If the thermal inertia of the rectum of the ram is the same as that of the calf[6], then changes in rectal temperature would lag behind changes in blood temperature by 5–10 min. This would mean that in these experiments the temperature of the blood supplying the brain starts to fall almost immediately after the onset of the polypnœa. Furthermore, the polypnœa was sustained in spite of the continued rapid fall in deep-body temperature, a response which is paradoxical since the air ambient to the rest of the body was cool.

Thus, these results support the view that a thermally induced polypnœa can be evoked in rams without a previous rise in central temperature. Moreover, they show that the fall in deep-body temperature which followed the increase in respiratory-rate did not extinguish the reflex while the scrotal skin was being heated. The nature of the receptors and their pathways have yet to be described. However, it is now apparent that in the ram the scrotal cutaneous receptors alone are capable of influencing general body temperature, giving a broader thermo-regulatory role to the scrotum than the purely local one previously considered[7,8].

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Polypnœa evoked by heating the Udder of the Goat

At the suggestion of Dr. Waites (preceding communication), we have similarly tested the udder of the female goat, because the sensory innervation of the udder and the scrotum are essentially the same[1].

In seven tests on 2 non-lactating pregnant Saanen goats, the immersion of the udder in a stream of air at 45° C. caused a rise in respiratory frequency from 20–40/min. to 100–120/min. after a latency of 5–60 min. Neither passing air at udder-skin temperature over the udder nor heating an equivalent area of the abdomen in front of the udder had such an effect. In two tests on a third dry, non-pregnant goat with one mammary gland transplanted on to the neck[2], the heating of this denervated gland was without effect on respiration, but when the control gland in the normal position was heated, the onset of panting occurred after 20 min. Although the rectal temperature tended to rise during the periods in the laboratory, the onset of polypnœa could not be related to this rise, which occurred equally in control experiments.

These preliminary observations confirm the finding of Waites that the inguinal region appears to be more sensitive to temperature than the general body surface of ruminants.

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Recovery of Fertility after Protracted Steroid-induced Sterility in Mice

Progesterone is known to prevent luteinization in experiments with guinea pigs[1]. The anti-luteinizing property of synthetic 19-norprogesterone and halogenated progesterone derivatives as absorbed from subcutaneously implanted pellets is greatly superior to that of natural progesterone[2,3]. The anti-luteinizing effect of these compounds can be made evident in experiments both with œstrogen-induced luteinization in ovarian grafts[4] and with spontaneous luteinization in the ovary *in situ* (guinea pigs[5], mice and rats). The anti-luteinizing effect of progestational compounds is not due to a direct action on the ovary but to a mechanism in which most probably the hypophysis and the hypothalamus are involved[6,7]. The physiological interest of these phenomena lies in the fact that the quantities absorbed per day from a subcutaneous pellet of progesterone and sufficient to antagonize luteinization are apparently not greater than those produced by the normal ovary *in situ*[4,5]. However, the findings are also of clinical interest; the progestational compound is also anti-ovulatory[8], in such a way that the anti-luteinizing action is implicitly also an anti-ovulatory one. Ample use of the anti-ovulatory action of progestational compounds has been made by Pincus *et al.* in experimental[10] and clinical[11] work.

The question arose whether the anti-ovulatory and anti-luteinizing faculty of progestational compounds can be made use of for the purpose of inducing protracted sterility and whether fertility can be recovered after a steroid-induced protracted sterility.

Mice, the life-cycle of which is such a short one and the reproductive activity of which deteriorates several months before death[12], are most suitable for such investigations. Our experiments were made with the strain *Balb-A* in use in this laboratory for several years; reproductive activity in this strain begins at the early age of two months, and is, under routine laboratory conditions, a stable one. But it starts to deteriorate 11–12 months later. Pellets of the highly active 19-norprogesterone were implanted subcutaneously; the pellets contained 20–40 per cent of this compound mixed with 80 and 60 per cent of cholesterol respectively.

20 per cent pellets were implanted into 10 mice 65 days old. The animals were killed 15 days later. Absorption was at the rate of 4–9 μgm. a day (for calculation of absorption from pellets containing a mixture of the specific steroid with cholesterol, see ref. 13). Corpora lutea were absent in 9 of these animals; the corpus luteum in the remaining animal was in a state of degeneration. In a group of 9 normal females 79 days old corpora lutea were present in 7 animals.

20 per cent pellets were implanted into 7 females 51–54 days old; 40 per cent pellets were implanted into another group of 7 females 40–51 days old. The experimental animals were maintained for the following 108 days with males in the same cage. None of these 14 animals became pregnant though they had reached the age of 5 months. In another group of 8 females 40 per cent pellets were implanted. The animals have now been sterile for 176 days and have reached the age of more than 7 months; that their sterility is not due to a failure of the male companion is shown by the fact that 2 other animals of the same group which lost implanted pellets had in the course of the same time 6 litters.

Table 1

No.	Age at implant. of pellets (days)	19-norprogest. per day (μgm.)	First litter after removal of sterilizing pellets (days)	Size of first litter after removal of pellets
20 per cent pellets				
5102	51	2	30	8
5094	54	2	30	11
97	51	2–6	no litter†	—
92	54	4–4	45	7
96	51	4–4	32	2
91	54	9•	31	10
93	54	9•	18(?)	9
40 per cent pellets				
5103	51	12	37	8
12	42	12	22	13
07	49	13	35	9
13	42	13	42	9
21	40	13	22	8
11	42	15	22	7
04	51	16	36	4

14 females *Balb-A* aged 40 to 54 days, implanted subcutaneously with two pellets of 19-norprogesterone. Pellets were removed 108 days later, when the animals were 5 months old. Males were present in the same cage from the beginning of steroid treatment. No pregnancy occurred before removal of pellets.

• Only 1 pellet found 108 days after implantation.

† *Added in proof.* First delivery 86 days after removal of pellets. Size of first litter, 5; subsequent deliveries of 11, 5 and 7.

Pellets were removed after having remained in the above 14 animals for 108 days. Absorption was at the rate of 2–4 μgm. a day with 20 per cent pellets, and of 12–16 μgm. with 40 per cent pellets. The animals were left with the same male as before. As seen from Table 1, 13 out of 14 animals became pregnant at 1–24 days after the removal of the pellets. The average number of young in the litter was 8, that is, no less than in the first litter of normal females of this strain which is of 6·7 (average of 15 normal animals). In one case (No. 5112) the size of the first litter after protracted sterility was even larger, reaching the maximum as observed in our normal animals.

In other words, after protracted steroid-induced sterility, full recovery of fertility occurred. The young were suckled as with normal litters; this was the case even with twelve young out of a litter of thirteen (No. 5112). The animals belonging to these litters began their reproductive activity at the normal age of about two months.

As mentioned above, the reproductive activity of females in *Balb-A* begins deteriorating at about the age of 13–14 months. It may be assumed that an experimentally induced sterility of three months in *Balb-A* mice corresponds to no less than five years in women.

Added in proof. The experiment in which 8 females with 19-norprogesterone remained sterile during 176 days has now also been concluded. All the animals remaining with the same male as during the sterility period became pregnant after the removal of the pellets and produced litters 20–42 days later. The average size of the litters was 6·25, 6·25 and 5·9 at the first, second and third delivery; that is, these females behaved as regards fertility like normal females. Six months of the reproductive life-span in our mice are assumed to be equivalent to not less than ten years in women.

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Arginine and 'Neurosecretory' Material in the Pituitary Gland of the Pig

IN a previous publication[1], the distribution of arginine as demonstrated histochemically by an improved Sakaguchi reaction[2] was examined in sections of rat pituitary gland. The results were discussed in relation to present knowledge of the origin and chemical nature of the hormones of the pituitary gland, with particular reference to the correlation between the stainable 'neurosecretory' material of the pars nervosa and the octapeptide antidiuretic-vasopressor hormone (ADH-vasopressin). It was further suggested that a similar examination of the neurohypophysis of the pig would be of interest since the ADH-vasopressin extracted from the posterior lobe of the pituitary of this animal contains lysine in place of arginine. Unfortunately, there does not appear to be a specific histochemical method for lysine; nevertheless, some information may be derived by applying the reaction for arginine, for if the stainable 'neurosecretory' material represents the hormones oxytocin and ADH-vasopressin, as postulated by the neurosecretory theory, then it might be expected to give no reaction for arginine in the case of the pig.

This communication describes results of such a preliminary investigation; pituitary glands were fixed in Susa or Lewitsky's fluid and the methods used were as described previously[1].

By comparison of adjacent sections it was found that the 'neurosecretory' material of the pars nervosa of the pig, as stained by the chrome alum-haematoxylin method, showed no reaction for arginine. However, other structures within the same section, such as cells of the pars intermedia, acidophils of the pars anterior, blood vessels and connective tissue fibres, gave a positive reaction for arginine, as did also 'neurosecretory' material in sections of rat pituitary stained under exactly the same conditions and used as a 'control'. It is therefore concluded that arginine is either very low in concentration or absent from 'neurosecretory' material in the pars nervosa of the pig, as predicted from the known chemical structure of pig ADH-vasopressin and the theory of neurosecretion. The investigation is being extended to other species.

I wish to thank Dr. F. R. Johnson, of the Department of Anatomy, for his help and advice on the photomicrography, and Mr. L. G. Cooper, of Messrs. T. Wall and Sons, Ltd., London, N.W.10, for providing the pig pituitaries, which were obtained within 30 min. of death.

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Renal Proliferation in the Unilaterally Nephrectomized Rat

RENAL growth following unilateral nephrectomy has been referred to as 'compensatory renal hypertrophy'[1]. The work of Rollason emphasizes the importance of hyperplasia in this process[2]. Utilizing thymidine as a specific precursor of deoxyribonucleic acid (DNA), and assuming[3] the constancy of DNA in the absence of dividing cells, the incorporation of thymidine serves as an index of cellular proliferation[3]. We now describe the effect of uninephrectomy on the incorporation of tritium-labelled thymidine into renal DNA.

Male Wistar rats, approximately 4 weeks of age and weighing 40 gm., were housed in colony cages and maintained on 'Purina Laboratory Chow' and water *ad lib.* Experimental and control groups were chosen at random from a group of sixty rats. Left nephrectomies and sham operations were performed under pentobarbital sodium anaesthesia (29 mgm./kgm. body-weight intraperitoneally). 36 hr. following the operative procedure each animal received intraperitoneally three separate injections, 1 hr. apart, of tritium-labelled thymidine, 20 μc./kgm. body-weight; 24 hr. following the last injection, the animals were killed by decapitation, the kidneys removed and frozen. DNA protein was isolated from the kidneys according to a modification of the Schmidt–Tannhauser procedure as outlined by Friedkin[4]. The dried DNA protein was weighed into a counting vial and 1 ml. of methanolic 'Hyamine' (Packard Instrument Co., Chicago, Illinois) was added. The vials were sealed and heated in a water-bath at 55° C. for 18 hr.; 5 ml. of a toluene scintillation solvent system were added[5]. A homogeneous solution was obtained by swirling. The vials were cooled to 4° C. and counted in a liquid scintillation counter (Packard Instrument Co.). The final counts were corrected for quenching by use of an internal standard. In order to express the counts as counts per min. per milligram of DNA, an aliquot of each DNA protein sample was heated with 3 per cent perchloric acid for 15 min. at 70° C., and the optical density of the supernatant solutions was read at 265 and 295 mμ and compared

Table 1

	No. of Animals	Average weight of total renal tissue (mgm.)	Per cent restoration of renal tissue	Thymidine incorporation (c.p.m. per mgm. DNA)	P
Nephrectomy	8	634	45	970 ± 84*	< 0·0001
Sham	4	877		488 ± 31*	

*Replicate determinations upon pooled tissue.

to a DNA standard (Sperm DNA, California Biochemical Co.).

A representative experiment is shown in Table 1. The unilaterally nephrectomized animals incorporated 100 per cent more labelled thymidine than did the control, sham-operated rats. These results, obtained with tracer amounts of tritium-labelled thymidine, are, according to our present concepts, indicative of increased cellular proliferation in the remaining kidney of the unilaterally nephrectomized rat[6]. They support the morphological work previously cited and recent reports concerning DNA synthesis in the uni-nephrectomized rat utilizing other techniques[7,8].

The mechanism(s) regulating such growth is poorly understood; however, Ogawa and Nowinski have reported a specific renal mitosis-stimulating substance in the serum of uni-nephrectomized rats[9]. We have found that kidney slices in a balanced salt–glucose medium incorporate tritiated thymidine into DNA at a measurable rate. Work is being carried out utilizing this *in vitro* technique as an assay for the presence of growth-promoting factors in the serum of unilaterally nephrectomized animals.

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The Distribution of Light in an Image formed in the Cat's Eye

Flamant[1] has estimated the distribution of light in the image formed in the human eye of a brightly illuminated slit. By the kindness of Prof. Arnulf and Mlle. Flamant of the Institut d'Optique in Paris, we were allowed to use their apparatus and method to make similar measurements on the eyes of cats. Visual stimuli have been used for electrophysiological work on the visual system of the cat, and it is often important to know how accurate an image is formed.

The cat was anaesthetized with 'Nembutal'; the pupil was dilated and the accommodation fixed with atropine. The image of a narrow slit of light formed in the cat's eye was photographed by the method described by Flamant[1] for the human eye. The eye was placed in Maxwellian view, so that a small image of the source was formed near the centre of the pupil, and an image of the slit at the tapetum. The light reflected from the tapetum was observed after emerging through the pupil, and the correcting lens was adjusted to make the tapetal image, as observed, as sharp as possible.

The principle of the method is to measure the increase in width of the photographic image of the retinal image of a vertical slit the intensity of which varied from top to bottom. It was immediately obvious that the image was much less sharp than that formed in the human eye. Satisfactory photographs were obtained from one cat only, but two others were examined and appeared to have images of similar size. When the image could be formed on the tapetum the reflexion was very much greater than that from the human eye, so that much shorter exposures were required.

The light which was photographed had traversed the eye media twice, once entering through a small central area of the pupil, and once leaving, after dispersion by the tapetum, through the whole of the dilated pupil. It is assumed that scattering in the second transit is equivalent to that occurring when the eye forms an image with the pupil dilated, and that scattering in the first transit is negligible in comparison. Fig. 1 thus represents the distribution of light in the retinal image of a point source when the light enters the eye through the whole of the dilated pupil. At 12 min. of arc from the centre of the image the intensity has fallen to about 50 per cent of its original value, at 32 min. the intensity is down to about 10 per cent. The spread of the image is about 6–8 times greater in these experiments than in those on the human eye. The conditions of our measurements were, however, different: the eye was atropinized and the position of the image on the cat's retina was not known—it was not necessarily very close to the central area. Comparison with human

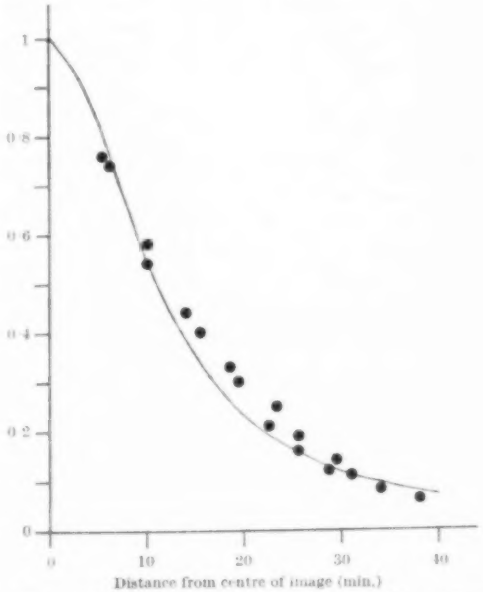

Fig. 1. Relative intensities at different angular distances from the centre of the image of a point source of light viewed by a cat's eye with pupil dilated

results may therefore be misleading. The experiments show that the images of small light sources formed by the cat's eye may be very blurred when light enters through the whole of the dilated pupil. They give no information about the scattering which takes place when light enters only through a small area in the centre of the pupil; this is likely to be much less.

We are grateful to Prof. Arnulf and Mlle. Flamant for allowing us to use their apparatus and for their assistance with the experiments. This work was supported by research grant *B*-1810 from the National Institute of Neurological Diseases and Blindness, United States Public Health Service, and both authors received Medical Research Council personal grants.

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A Neuronal Model for Conditioning

THE difficulty of explaining conditioning in terms of a simple neuronal model has been discussed by Burns[1]. The problem lies in the fact that the only relatively long-lasting type of facilitation that has so far been experimentally demonstrated involves presynaptic terminals so that activity of neurones in the unconditioned reflex pathway cannot be expected to facilitate synaptic connexions from neurones which converge on to them from another afferent pathway. Eccles has proposed a rather complex neuronal model to avoid this difficulty[2]. Even this is admitted to be inadequate since it requires that the conditioned and unconditioned stimuli be applied simultaneously, and even then could only provide a low-level bombardment of the synapses which must be facilitated unless additional reverberating circuits are included. There is evidence to suggest that reverberating circuits do play a part in the early stages of conditioning[3,4], but no complete model including them has been proposed.

The following simple scheme appears to fulfil many of the requirements: the neurones of the unconditioned reflex pathway are linked to the afferent neurones excited by the conditioning stimulus through a number of parallel reverberating circuits. Each unit (Fig. 1) is reciprocally linked through synapses with similar parallel units. The synaptic resistance must be such that impulses from *C.S.* alone result in the damped oscillation of only a few of the units. If impulses from *U.S.* impinge on the circuits while damped oscillations initiated by *C.S.* are still present, then spatial facilitation occurs and additional parallel circuits are recruited. Once a critical number of parallel units is activated the oscillations become self-maintaining because of reciprocal reinforcement. When the oscillations eventually die down the synapses linking *C.S.* to *E* through *A*, *B* and *C* remain facilitated.

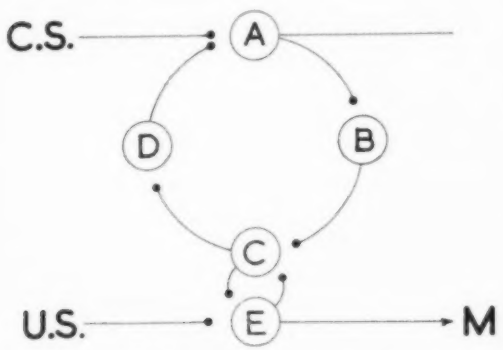

Fig. 1. *A*, *B*, *C*, *D* and *E* represent nerve cell bodies in a model unit. *C.S.* is the afferent pathway for conditioned stimuli and *U.S.* for the unconditioned stimulus. *M* represents the motor pathway for the unconditioned response

A difficulty arises. *E* is continuously bombarded with impulses during the initial stage of the conditioning process which may be expected to prolong the reflex greatly. It seems possible that activity in *E* may be cut off by negative feedback once the reflex has been fulfilled.

I thank Dr. B. D. Burns for his comments.

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Local Endotoxin Hypersensitivity and its Relation to the Shwartzman Phenomenon

ALTHOUGH there have been previous claims that lesions like the Shwartzman phenomenon can be produced by two large injections of endotoxin into the same site in the skin, in animals other than rabbits (for example, mice)[1], it has been stated that the classical Shwartzman phenomenon is immunologically non-specific in that it does not depend on antigen antibody reaction[2,3], and it can be elicited only in rabbits[2-4]. Recently, however, I have succeeded in demonstrating that the first phase of the Shwartzman phenomenon, that is, preparation of the skin by endotoxin, is a specific reaction depending on a well-characterizable immunological mechanism, namely, the phenomenon of local endotoxin hypersensitivity, and that in appropriate circumstances (*a*–*c*) it may be elicited by endotoxins on the skin of guinea pigs and rats also.

These characteristics are:

(*a*) Guinea pigs and rats, 3–4 weeks previously sensitized with *S. typhosa* or *E. coli*, will produce hemorrhagic skin lesions at sites prepared with 25–50 μgm. endotoxin, on intravenous injection of 50–100 μgm. endotoxin 24 hr. later. (The sensitization was carried out by the weekly subcutaneous injection of 0·5–1, 0·1, 0·2 × 10^{-9} bacilli. Typhoid and *coli* endotoxin prepared by the Boivin method were reprecipitated three times by a double volume of ethanol. Their Shwartzman-reactive unit was 3 μgm.) Animals not pretreated with the bacteria do not produce hemorrhagic lesions. These experiments proved that the endotoxin must not be derived from the same bacterial species as was used for sensitization.

(*b*) 0·025–0·1 ml. rabbit antiserum, obtained by hyperimmunization with typhoid endotoxin, injected

into the skin of guinea pigs or rats, followed after 24 hr. by intravenous endotoxin, results in skin haemorrhage.

(*c*) Mixture of typhoid endotoxin (25–50 μgm.) with colloidal silver (50–100 μgm.) for intracutaneous injection and subsequent intravenous challenge (0·1–1·0 μgm. endotoxin and 10–20 mgm. colloidal silver) results in skin haemorrhage in guinea pigs at 6–8 hr.

(*d*) Normal rabbit mononuclear peritoneal exudate cells ($2–10 \times 10^4$) obtained 4 days after paraffin oil had been introduced in the peritoneal cavity[5], injected intracutaneously in guinea pigs, and followed by 50–100 μgm. endotoxin intravenously after 24 hr., result in skin haemorrhage.

(*e*) Normal guinea pig or rat mononuclear peritoneal exudate cells ($5–10 \times 10^4$) injected intracutaneously in guinea pigs or rabbits, and followed by 100 μgm. endotoxin intravenously after 24 hr., result in skin haemorrhage.

The histology of the skin lesions (6–8 hr. after intravenous challenge) resembled that of the Shwartzman phenomenon in rabbits.

a–c could be explained by localization of endotoxin in relatively high concentrations in the skin by the antibody or by the effects of colloidal silver. Antibody which combines with endotoxin has been shown (in some instances at least) not to neutralize its toxic properties. A specific additional effect of antigen–antibody reaction or of colloidal silver might or might not be involved, but it does not seem proved.

d and *e* can scarcely be explained in the same way, since the peritoneal exudate cells do not produce secreted antibody in normal circumstances. The possibility is worth examining that they carry delayed type hypersensitivity to endotoxin, and that the combined result of a delayed type reaction and intravenous endotoxin is a Shwartzman phenomenon.

I wish to thank Dr. E. Kelemen for advice and generous help throughout this work.

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Lactic Acid Concentration in the Kidney of the Golden Hamster, *Mesocricetus auratus*, in Different Conditions of Diuresis

Work by Zerahn on frog skin[1] and by Leaf on the bladder of the toad[2] have produced evidence that the active transport of sodium is accompanied by a proportional consumption of oxygen; glycolysis and lactic acid production also increase, according to Leaf, with the active transport of sodium in aerobic conditions.

It is known that the renal emunctory is also a site of intense transfer of sodium through the convoluted and collector tubes from the urine to the plasma; in addition, it seems that consumption of oxygen is related to such a process[3]. In the present communication we present the results obtained on lactic acid concentration in the cortex and papilla of the kidney in three different experimental conditions: (1) after 12 hr. fasting; (2) under water diuresis (samples of renal tissue taken 1½ hr. after the administration of distilled water to the amount of 5 per cent of the animal's weight by gastric tube); (3) under mannitol diuresis (samples of renal tissue taken 1½ hr. from the subcutaneous administration of 7·5 ml. per cent body-weight of a 10 per cent mannitol solution).

Mesocricetus auratus was selected for experiments as the renal cortex of its kidney can be easily separated from the papillary tissue (Fig. 1). The lactic acid has been measured by Barker and Summerson's method[4] using as a standard lithium lactate purified according to Schatzkes[5]. Each examination was made on samples of approximately 40 mgm. of papilla tissue and 100 mgm. of cortical tissue, frozen in liquid nitrogen as soon as they were taken from the animal; the two samples of cortex and papilla which were compared came from the same kidneys.

Fig. 1

The results are shown in Table 1. From these a constant and significant difference of the lactic acid content in the papilla and in the cortex appears in all conditions. If one excludes a state of relative hypoxia in the papillary zone, this difference could account for greater chemical work in the papilla than in the cortex, or it could simply be the expression of a greater concentration in the tissue of this part as there is for sodium chloride and urea[6].

Table 1. Lactic Acid mgm. per cent gm. of Fresh Tissue

Treatment	Cortex mean value	Papilla mean value	Difference ± S.E.
$n = 7$ Water diuresis	50·93	71·12	20·19 ± 3·55
$n = 7$ Mannitol diuresis	54·25	80·29	26·03 ± 3·48
$n = 8$	54·00	108·88	54·88 ± 9·91

It is interesting that under mannitol diuresis the difference increases significantly, although it seems that the cortex–papilla gradient of total concentration tends to decrease in this condition[7]; the greater concentration of lactic acid in the papilla under

mannitol diuresis seems therefore to exclude the second hypothesis.

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Pharmacological Actions of Some Methoxyindolealkylamines

The O-methylation of catecholamines has been shown to be an inactivation process[1], but the recent characterization of melatonin (N-acetyl-5-methoxytryptamine—a potent agent in lightening frog skin) as a methoxy indole[2] indicates that this may not be true with hydroxyindolealkylamines. To investigate this possibility, we have compared the actions of a number of methoxyindolealkylamines with their corresponding analogues on smooth muscle, blood pressure and behavioural patterns.

Oxytocic activity was measured on isolated oestrous rat uterus. Gramine and its methoxy derivative were found to possess no oxytocic activity at a dose-level as great as 0·1 mgm. per ml. (Values are given as final concentration in the muscle bath.) At the same dose-level, 5-hydroxyindole acetic acid and 5-methoxyindole acetic acid caused small but demonstrable contractions. This action could not be blocked with bromolysergic acid diethylamide (*BOL*). N-Acetyl-5-methoxytryptamine also exhibited slight oxytocic effect, but only at the high dose-level of 0·1 mgm. per ml.

The four other compounds tested were serotonin, bufotenine and their respective methoxy analogues. These exhibited marked oxytocic activity at dose-levels below 10 μgm. per ml. The relative activity of these compounds is given in Table 1, and it can be seen that the methoxy derivatives were only slightly less active than their hydroxy analogues. The results obtained for bufotenine and 5-methoxytryptamine are in general agreement with those of Erspamer[3] and of Barlow and Khan[4].

Whereas N : N-dimethylation of 5-methoxytryptamine only reduces the oxytocic activity slightly, N-acetylation reduces the potency a thousand-fold.

The effect on arterial blood pressure was measured on a smoked drum from a mercury manometer. Two cats and three dogs anæsthetized with pentobarbital were used. The action of compounds was noted before and after the nervous system had been destroyed in cats by pithing, or blocked in dogs by large doses of the ganglioplegic agents : tetraethyl ammonium chloride, 30–40 mgm./kgm., or chlorisondamine ('Ecolid' : 2 mgm./kgm.).

In doses of 1–2 mgm. N-acetyl-5-methoxytryptamine, 5-hydroxyindole acetic acid, 5-methoxyindole acetic acid and 5-methoxygramine had no effect on arterial blood pressure of dogs or cats.

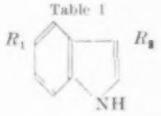
Serotonin, 5-methoxytryptamine, 5-methoxy-N : N-dimethyltryptamine in doses of 0·1 mgm. and bufotenine, 0·5 mgm., all caused depression of arterial pressure or an amphibaric response in both species while the nervous system was intact. After pithing or blocking, these compounds caused blood pressure to rise by 26–92 mm. mercury.

It has been reported that ganglioplegic agents cause powerful augmentation of a variety of pressor agents[5]. Serotonin and 5-methoxy-N : N-dimethyltryptamine were augmented to an almost identical degree, both being slightly exceeded by 5-methoxytryptamine. 5-Methoxytryptamine also exhibited a depressor component, after pithing in cats, usually following a strong pressor phase.

The relative vasopressor potency of these four compounds is : Serotonin > 5-methoxy-N : N-dimethyltryptamine > 5-methoxytryptamine > bufotenine. Thus, O-methylation reduces the vasopressor activity of serotonin but greatly increases that of bufotenine, and N-acetylation of 5-methoxytryptamine inactivates it.

To investigate behavioural disturbance, rats were conditioned to escape, in a shuttlebox, from a 535-V.

Table 1



Compound	R_1	R_2	Oxytocic*	*BOL*†	Effect on blood pressure‡ (unblocked)	Effect on blood pressure‡ (blocked)	Behavioural§ disturbance
Gramine	H	CH_2NH_2	0·0001	—	None	None	0
5-Methoxygramine	OCH_3	CH_2NH_2	0·0001	—	,,	,,	+
Serotonin	OH	$CH_2CH_2NH_2$	1	Inhib.	⇅	↑	(++)
5-Methoxytryptamine	OCH_3	$CH_2CH_2NH_2$	0·7	,,	⇅	↑	++
N-Acetyl-5-methoxytryptamine	OCH_3	$CH_2CH_2NHCOOH_3$	0·0001	—	None	None	0
5-Hydroxyindole acetic acid	OH	CH_2COOH	0·0001	No inhib.	,,	,,	—
5-Methoxyindole ,, ,,	OCH_3	CH_2COOH	0·0001	,,	,,	,,	—
Bufotenine	OH	$CH_2CH_2N(CH_3)_2$	0·49	Inhib.	↑	↑	++
5-Methoxy-N : N-dimethyltryptamine	OCH_3	$CH_2CH_2N(CH_3)_2$	0·08	,,	⇅	↑	+++++

* Potency of equimolar concentrations of the base. † Inhibitory effect of *BOL*. ‡ Blood pressure raised ↑, biphasic ⇅. § At 0·1 m*M*/kgm. dose-level each + represents 2 mistakes during 10 tests.

2-m.amp. shock in response to a warning auditory stimulus, individual trials being separated by 6-min. intervals. The criterion of completed conditioning was a correct response to the conditioning stimulus of not less than 9 out of 10 times on two consecutive days. The animals were then used for assay and the number of mistakes plotted against the dose-level. N-Acetyl-5-methoxytryptamine caused no behavioural disturbance at dose-levels of 0·2 mM/kgm. At dose-levels between 0·1 and 0·3 mM/kgm. 5-methoxygramine caused a somewhat greater degree of disturbance than did gramine. At 0·1 mM/kgm., 5-methoxytryptamine, bufotenine and serotonin all gave similar results, though in the case of the latter compound the paresis of the hind legs which developed introduced a physical handicap which modified the interpretation of this result. The most active compound of the series proved to be 5-methoxy-N : N-dimethyltryptamine, which caused 10 mistakes out of 10 trials at a dose-level of 0·05 mM/kgm. This was three times more effective than bufotenine, which has been reported to be psychotomimetic in man[6] and to affect the performance of trained rats[7]. At dose-levels exceeding 0·1 mM/kgm. the animals given 5-methoxy-N : N-dimethyltryptamine developed rhythmic beating of the forepaws, which tallied closely with the description given by Fabing and Hawkins[8] for the effects of bufotenine in rats which we have, however, not observed. 5-Methoxytryptamine also proved quite potent and in the same range as bufotenine. Finally, 5-methoxygramine appeared to cause more mistakes than did gramine itself.

Thus the ability of gramine, serotonin and bufotenine to cause behavioural mistakes was increased by O-methylation; and whereas N : N-dimethylation of 5-methoxytryptamine increases its potency, N-acetylation inactivated it.

It can therefore be concluded that O-methylation of hydroxyindoles is not an inactivation mechanism as is the O-methylation of catecholamines. The O-methylation of hydroxyindoles, however, changes their activity in the three assay procedures used in the following ways: oxytocic activity is still exhibited though slightly decreased; the vasopressor activity of serotonin is decreased but that of bufotenine is increased; ability to cause behavioural mistakes is increased. In addition, although O-methylation and N-acetylation are both necessary for the frog skin lightening activity of melatonin[9], it is the N-acetyl group which renders melatonin inactive towards smooth muscle, blood pressure and behaviour.

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HÆMATOLOGY

A Possible Specificity of Albumin Auto-antibodies

Anti-*I* was the name given by Wiener *et al.*[1] in 1956 to a cold antibody found in the serum of a patient suffering from cold antibody haemolytic anaemia. Further investigations into the *I* group system were made by Jenkins *et al.*[2], Tippett *et al.*[3], Weiner *et al.*[4] and Marsh[5]. These workers showed that cold auto-antibodies (previously called non-specific cold agglutinins) had *I* specificity and that occasional powerful examples of these antibodies may be found as auto-agglutinins in cases of cold antibody haemolytic anaemia. The *I* antigen has an almost universal distribution. Wiener *et al.* finding only five *i* people from 22,000 blood donors, while Jenkins *et al.* failed to find an example of the *i* phenotype after testing 17,000 donors. Marsh afterwards described examples of cold auto-antibodies having *i* specificity and showed that normal infants at birth possessed the *i* phenotype, which changed within 18 months of birth to *I*. He concluded that the rare *i* phenotype in adults arose from the absence of a genetically determined factor essential for the proper development of *I*. The majority of cold auto-antibodies therefore appear to have *I* specificity, a minority may be anti-*i*.

In 1956, Weiner *et al.*[4] described some examples of a rare auto-agglutinin active against all cells, but only when they were suspended in bovine albumin. These auto-agglutinins were inactive by anti-globulin or enzyme techniques and could not be absorbed by saline-suspended red cells. We have encountered two examples of the albumin auto-agglutinin described by these workers and were able to test them for *I* or *i* specificity.

Both sera were active against the patient's own cells and also all cells of a large genotyped panel, but only when albumin suspended red cells were used. One serum contained an additional trace of anti-*D* which was successfully removed by absorption with saline-washed rhesus-positive red cells.

The sera were tested against the red cells of two examples of the i_1 phenotype, one i_2 and four cord blood samples, with appropriate adult controls. The tests were all positive with about the same strength of reaction. Repeated tests with one of the sera in titration (using *AB* serum as a diluent) showed that the i_1 and i_2 cells scored the same as the adult controls; the cord samples were slightly weaker.

It is apparent from these results that while cold auto-antibodies have *I* or *i* specificity, albumin auto-agglutinins are not aimed at antigens within the *Ii* system.

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Action of Proteolytic Enzymes on the Human Erythrocyte Surface

SEAMAN *et al.*[1] have recently described the liberation of a sialomucopeptide by the action of trypsin on the human erythrocyte surface. Similar results have been obtained by Mäkelä *et al.*[2]. My work concerned with the chemistry of *MN* blood group-active mucoids from human erythrocytes has revealed that N-acetylneuraminic acid-containing mucoids, which carry *MN*-active groupings, were liberated into the supernatant by the action of papain[3]. A review of the *MN* blood group active substances, their isolation and inactivation by several methods has been published elsewhere[4]. Further work led to the examination of other enzymes which are known to inactivate *M* and *N*: trypsin, ficin, bromelin, and stem-bromelain[4]. In all cases the isolation procedure to recover the non-dialysable split products was similar to that described previously[2] for papain-treated red cells, which involved phenol/extraction of the supernatant after incubation with enzyme and centrifugation. Some chemical data of the mucoids obtained are given in Table 1.

Table 1. QUANTITATIVE CARBOHYDRATE VALUES OF MUCOIDS RELEASED BY DIFFERENT ENZYMES

	Trypsin	Ficin	Bromelin	Papain
N-Acetylneuraminic acid (per cent)	19	18	13	14·7
Reducing sugar (galactose) (per cent)	29·4	39·9	73·5	47
Hexosamine (per cent)	9·9	13·9	8·2	18·1

In the case of bromelin and also stem-bromelain a glucose-containing polysaccharide from the crude enzyme is recovered in the final product so that chemical analysis gives carbohydrate values which are too high. Paper chromatography showed in all other samples the presence of galactose, glucosamine, galactosamine and small amounts of fucose and mannose. The same sugars have been detected in other *MN*-active preparations from erythrocyte stroma[3]. Although these mucoids were isolated from human *O* cells, no *H*-activity could be demonstrated by rabbit anti-*H*; only slight inhibition was observed when tested with anti-*H* extract from *Ulex europaeus*.

The fact that all N-acetylneuraminic acid-containing and *MN* blood group-active mucoids prepared in this way give no inhibition of myxovirus haemagglutination has been interpreted[4] and may be in accord with the findings that the enzyme-treated cells are equally well agglutinated by mumps- and influenza-virus as are the untreated cells. The *MN* activity of these substances is considerable but not so good as the activity of preparations isolated by a procedure where no enzyme is involved[4]. By the latter method large amounts of the N-acetylneuraminic acid-containing influenza-virus receptor are also obtained[4]. In the light of these observations the lowered electrophoretic mobility of enzyme-treated cells is understandable. On the other hand, it is worth noting that removal of either neuraminic acid or neuraminic acid-containing mucoids renders the cells agglutinable by incomplete antibodies[2,4].

The same method has been successfully used for investigating mucoids released by papain from animal erythrocytes. The substance from bovine erythrocytes has already been described[3,4]. The material from horse cells contains 15 per cent *N*-glycolylneuraminic acid, 21 per cent reducing sugar, 5·7 per cent hexosamine and inhibits slightly the agglutination of human *O*, *N* cells by anti-*N* extracts of *Vicia graminea*; whereas that from hog cells has 9·5 per cent N-glycolylneuraminic acid, 33·5 per cent reducing sugar and 11·2 per cent hexosamine. Tests of the treated cells with mumps- and influenza-virus indicated that not all the neuraminic acid-containing mucoids had been removed[4].

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Blood Potassium and Sodium of Hereford and Brahman Cattle and some Breeds of Sheep maintained in Florida

INCIDENTAL to other comparative physiological investigations with taurine and zebu cattle[1], it was possible to determine the potassium and sodium content of the erythrocytes of these species by flame spectrophotometry. Although there have been several recent reports concerning potassium and sodium in the blood of sheep and their interesting genetic implications[2], there is a paucity of information regarding these elements in the red blood cells of cattle.

Abderhalden was one of the first to study blood electrolytes of cattle, and more recently Kerr reported findings for six *Bos taurus* cattle. The low potassium and relatively high sodium of the erythrocytes of cattle compared with other species as found by Kerr and Abderhalden have been widely cited in text-books, in spite of their meagre basis. Bernstein[3] has, however, confirmed the results of the earlier workers, but fails to state which types of cattle were used in his work.

Twelve Hereford and twelve Brahman heifers in the sixth or seventh month of gestation, and all approximately the same age, were used in the present work. All animals were under the same management and were kept under identical environmental conditions. All the animals were on an adequate plane of nutrition as described previously[1]. Blood was collected in heparinized tubes from the jugular vein of each of these twenty-four animals and centrifuged under standard conditions with a centrifugal force of 1,500*g*. The force was applied for 2 hr. rather than 30 min. in order to eliminate more of the plasma entrapped between the packed cells. Jennings *et al.*[4] have reported that bovine blood centrifuged under standard conditions for 30 min. still retains some 12·5 per cent of the plasma in the packed cell column; but Chaplin and Mollinson[5] were able to remove most of the trapped plasma by increasing the time the centrifugal force was applied.

A 1-ml. sample of plasma was first taken from each tube of blood, then the remainder of the plasma and the upper layer of cells were removed and discarded. A small quantity of unwashed erythrocytes were then placed in a clean weighed volumetric flask which was reweighed, and sodium determinations were carried out using a Beckman model *B* spectrophotometer at wave-lengths of 770 and 585 mμ respectively. All results were corrected for dilution and those for the erythrocytes adjusted for weight

Table 1. Potassium and Sodium Content of Bovine Erythrocytes and Erythrocytes and Plasma expressed as m.equiv./l. for Hereford and Brahman Heifers

	Herefords				Brahmans			
	Erythrocytes		Plasma		Erythrocytes		Plasma	
	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+
1	16·5	118	9·0	146	14·1	115	6·4	146
2	18·0	107	6·4	141	15·7	127	6·4	141
3	16·8	104	7·7	141	11·2	91	6·4	137
4	13·9	124	10·2	146	11·9	102	8·3	146
5	15·7	112	7·7	141	17·2	130	6·4	137
6	20·4	113	10·2	146	12·9	202	6·4	141
7	15·7	118	5·1	130	15·1	95	9·0	141
8	25·7	119	9·0	141	11·3	73	7·7	141
9	14·6	103	9·0	141	20·7	125	7·7	135
10	17·0	108	7·7	137	14·7	154	6·4	135
11	18·6	109	9·0	137	11·7	129	7·0	137
12	27·6	116	11·5	141	8·5	134	6·4	135
×	18·4	113	8·5	141	13·8	123	7·0	140

$$\text{Hereford } \frac{[K^+]_I}{[K^+]_E} = 2{\cdot}16 \qquad \frac{[Na^+]_I}{[Na^+]_E} = 0{\cdot}80$$

$$\text{Brahman } \frac{[K^+]_I}{[K^+]_E} = 1{\cdot}96 \qquad \frac{[Na^+]_I}{[Na^+]_E} = 0{\cdot}88$$

Where $[K^+]_I$ = intracellular potassium and $[Na^+]_E$ = extracellular sodium

of sample and red cell specific gravity (1·088). All the findings are presented in Table 1 as m.equiv./l.

The Herefords appear to have more potassium and less sodium in their erythrocytes compared with Brahmans, the differences for the potassium being significant at the 1 per cent level. Apart from this, these results further confirm the bovine characteristic of low potassium and high sodium content in red cells. The differences in the plasma electrolytes are small and not significant. The ratios of intracellular to extracellular sodium are similar to those reported by Bernstein[3], but the potassium ratios are slightly lower. Bernstein has shown that the potassium concentration ratio for red cells and blood plasma of the dog and cat is approximately 2, and implies that this small ratio may be explained on the basis of the Donnan equilibrium. The higher concentration ratios for potassium obtained by Bernstein for other species would, however, require extra energy for maintenance. The small differences in the potassium concentration ratios by Bernstein and those reported here could be explained by the differences in preparing the red cells for analysis. It has also been demonstrated that the potassium content of erythrocytes varies to a small degree with the level of dietary potassium[4].

Red cells from 35 ewes were similarly examined for potassium and sodium. Five of these animals were Hampshires, 20 Rambouillets and 10 Florida native ewes. The last-named represent a breed which has developed largely by natural selection in Florida since the time of early White settlement. Only a relatively small population of this breed exists, and it closely resembles a degenerate Cheviot, being much smaller and having an inferior coat when compared with that breed. Doubtless the Cheviot has figured in the background of this local breed; but it is also highly likely that other breeds have been intermingled at various times. Compared with imported breeds in the hot, humid environment of Central Florida these native sheep are much more hardy and more tolerant of the usual parasitic and disease conditions associated with sheep in moist places. The Florida native sheep is also characterized by a significantly higher blood hæmoglobin than imported breeds maintained under similar conditions[7].

Despite a well-defined seasonal pattern in blood hæmoglobin of sheep kept in Florida, the native always has a significantly higher concentration[7].

The blood of sheep has been classified into three different types by Kerr and Evans[2] on the basis of erythrocyte potassium. According to Evans, European sheep possess for the most part only two of these types, but Kerr found the third type well represented in Lebanese fat-tailed sheep. Evans has designated the two types common in European sheep as (*L K*), low potassium, and (*H K*) high red cell potassium. For the most part Evans has analysed whole blood to distinguish the potassium-levels, assuming that plasma potassium is fairly constant. While this is probably true, hæmatocrits vary, particularly in exotic livestock maintained in the lower latitudes. The chances of anæmia due to increased parasitic infestation, lower plane of nutrition or certain types of climatic stress are greatly increased, and it was therefore thought to be more reliable to conduct the analyses for red cell potassium directly on the erythrocytes.

Table 2. Incidence of the (*H K*) Blood Type in Three Breeds of Sheep Maintained in Florida and a Comparison with Other Results

	5 Hampshires (per cent)	20 Rambouillets (per cent)	10 Florida natives (per cent)
H K	25	5	30
Evans (ref. 2)	28·7	0	(29·1 Cheviot)

The results for the few sheep examined are outlined in Table 2 and would not be expected necessarily to compare too favourably with other results, due to the small numbers. However, these results agree fairly well with those of Evans and co-workers, and it is interesting to note that the incidence of the *H K* blood type in Cheviots as found by Evans closely resembles that for Florida natives.

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PATHOLOGY

Mechanism of Increase of Endotoxin Susceptibility in Animals showing Delayed Hypersensitivity

In recent years considerable attention has been paid to the fact that an increase in susceptibility to the pyrogenic and shock-producing action of bacterial endotoxins could be demonstrated in animals showing delayed hypersensitivity[1].

Chinchilla rabbits were sensitized intravenously with live B.C.G. vaccine at a relatively high dose of 10–20 mgm. wet weight. Their condition was assessed repeatedly within 3–8 weeks after sensitization. We measured the variations of body-temperature during the day, skin hypersensitivity to 2, 0·4, 0·08, and 0·016 μgm. purified protein derivate and systemic hypersensitivity to 5 μgm. purified protein derivate or 20 μgm. heat-killed and twice washed B.C.G. bacilli injected intravenously. The increased degree of susceptibility to the pyrogenic effect of purified[2] *Salmonella paratyphi B* endotoxin was evaluated by determining the threshold doses capable of provoking standard fever reactions. In four independent experiments altogether 61 rabbits were used, and more than 30 normal unsensitized rabbits were used as controls. Only absolutely healthy animals were used, and allowance had to be made for the influence of the season of the year.

Controls generally gave the following results: no skin reactions to the tuberculin test doses, no fever reaction to 5–50 μgm. purified protein derivate and 20 μgm.–5 mgm. doses of killed B.C.G. given intravenously. Initial morning temperatures in the majority of the animals were 39° ± 0·2° C.; afternoon and evening temperatures remained unchanged or increased by less than 0·5 deg. C. Normal fever reaction was approximately + 1 deg. C. within the first 2 hr. after the injection of 1 ml. endotoxin in 10^{-8} dilution.

With test rabbits sensitized with the B.C.G. vaccine, the following results were obtained: different degree of skin hypersensitivity; positive systemic reaction in 62 per cent, negative in 38 per cent, of cases. In general, quite good correlation was observed between the results of the skin and systemic tuberculin reactions.

In 32 per cent of test rabbits the initial body-temperature rose by more than 0·5 deg. C., sometimes even by 1 deg. C., as compared with the average temperatures in controls. In approximately two-thirds of test animals the variations in day-temperature were greater than 0·5 deg. C.; in a quarter they exceeded 1 deg. C.

The pyrogenic endotoxin reaction in 45 per cent of test animals was similar to that in controls; a stronger reaction was noticed in 55 per cent of the animals. In these cases the standard fever reaction was produced by endotoxin doses 2–5 times lower than in control rabbits.

By comparison of all the results from individual animals, we were able to distinguish three well-defined groups among the test rabbits. The first group was characterized by a marked skin hypersensitivity, positive systemic reaction to tuberculin and killed B.C.G., normal initial body-temperature and daily temperature rhythm, and normal endotoxin reaction. A contrasting group consisted of animals exhibiting small skin hypersensitivity, negative systemic reaction, increased initial temperature as well as daily temperature variations, and increased endotoxin susceptibility. The third group gave intermediate results.

After B.C.G. sensitization, the development of specific hypersensitivity, accompanied by non-specific increase of endotoxin susceptibility, would be expected in all rabbits; yet this was not found. The increased susceptibility to endotoxins was not correlated with the degree of specific hypersensitivity, but quite otherwise. This excludes the hypothesis that the systemic tuberculin reaction is not a specific hypersensitivity reaction but that it should be considered as a reaction to traces of endotoxic substances present in tuberculin[3]. Moreover, this hypothesis is also refuted by the quantitative relations observed.

The increased endotoxin susceptibility might be attributed to the summation of irritations which arise in the reaction of hypersensitive cells with the sensitizing antigen persisting in the body. In the course of such a reaction, a partial desensitization of cells takes place and substances possessing pyrogenic and other effects are released[4]. In consequence, the state of unbalanced thermoregulation and increased susceptibility to endotoxins develops, together with a decrease in specific hypersensitivity. In animals where these reactions did not take place, a high degree of specific hypersensitivity is observed, without the non-specifically increased susceptibility. This hypothesis was confirmed in further experiments in which various sensitization doses were used[5]. Specific hypersensitivity and increased endotoxin susceptibility do not appear to be two parallel manifestations of delayed hypersensitivity.

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Absence of Thyroid Auto-antibody in Experimental Radiation Thyroiditis

The association between thyroiditis and thyroid-specific autoantibody, both in man[1] and in animals[2], was established in 1956. There is still controversy as to whether the autoantibody is the cause or the consequence of the thyroiditis or whether, indeed, both thyroiditis and autoantibody are independent results of a single pathogenetic factor. However, it is widely believed that escape of thyroglobulin from the thyroid follicles initiates the development of autoantibody. It has been suggested that in experimental thyroiditis induced by radioactive iodine, the inflammation is a response to the liberation of colloid in the interfollicular stroma[3,4]. We have administered radioactive iodine to guinea pigs in order to determine whether autoantibody is produced in these circumstances. It is known that the guinea pig readily produces thyroid autoantibody when injected intradermally with thyroid extract[5].

Thirty-six adult female guinea pigs were divided into three groups, which were given single intraperitoneal injections of 0·2, 0·4 and 2·0 mc. of iodine-131 per kgm. body-weight, while three further animals received saline injections only. Eleven of the animals given the two lower doses received an additional injection of 8 mc. per kgm. 56 days later, in an attempt to stimulate a secondary antibody response when a primary response was undetectable by the technique used. Sera were tested for tanned cell agglutinating thyroid autoantibody[6] at intervals

from one day to 229 days after injection. Animals were killed at intervals, and their thyroids examined histologically.

Those animals given the two lower doses showed no histological evidence of thyroiditis up to 28 days after injection. Animals given 2 mc. per kgm. showed no abnormality of the thyroid when examined from 1 to 5 days after injection, but thyroiditis was present from 7 days onwards. With a dosage of 8 mc. per kgm., there was mild thyroiditis when the animals were first examined at 4 days, progressing to extensive necrosis at 7 days. At this stage, the histological picture in those areas which were not necrotic was that of disruption of the follicles, with intermingling of epithelial cells, inflammatory cells and fibroblasts. With such disorganization of the gland there could be little doubt that colloid had been liberated from the follicles. In the later stages the inflammatory reaction subsided and there was considerable regeneration of thyroid epithelium. The high dose of iodine-131 required to produce thyroiditis is due to the low iodine uptake on diet *S.G.*1 (Oxoid).

In none of the 138 sera examined was there detectable autoantibody. Thus, in spite of the presumed escape of thyroglobulin from the follicles at an early stage in the experiment, the development and regression of thyroiditis occurred without production of autoantibody.

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BIOLOGY

Molecular Biology or Ultrastructural Biology?

It is generally recognized that in the past decade or so we have seen great advances, which are of inestimable value for our understanding of fundamental biology, in the field which is concerned with proteins, nucleic acids, genes, viruses, antibodies, enzymes, etc. It is gratifying to note that several important bodies which are in a position to influence public policy in scientific matters have singled out this field as one requiring increased support.

It is unfortunate, however, that it seems to be becoming usual to refer to this connected series of topics by the name 'molecular biology'. This phrase is only marginally appropriate to any aspect of this area of biology, and is highly inappropriate to the greater part of it. Its most natural application would be to the biology of molecules of the conventional chemical kind, such as those involved in intermediate metabolism, and these are precisely not the types of entity with which the field under discussion deals. The smallest particles with which the new advances have to do may, indeed, be regarded as falling near the upper limit of size of the molecular, and, as for some proteins for example, are open to investigation by, more or less, conventional 'molecular' or chemical techniques. The greater part of the recent advances, however, have dealt with entities which are not defined in chemical terms; for example, genes, cistrons, mutational sites, ribosomes, antibodies, endoplasmic reticulum, and so on; and they have been studied predominantly by such non-chemical methods as those involved in viral and bacterial genetics, induced enzyme synthesis, complementation studies, immunology, ultra-centrifugation, electron microscopy, X-ray spectroscopy, and the like. An attempt to squeeze the whole of this wide front of advance under a title such as 'molecular biology', which places the emphasis on physical and chemical methods of investigation, brings with it the danger that the equally essential biological methods—which are in some respects even more penetrating than our existing chemical methods—will be unduly neglected.

The general characteristic of the field under discussion is, surely, that it is concerned with the analysis of entities, such as genes and ribosomes, which fall in the scale of magnitude lying between those visible with the light microscope and those accessible to conventional biochemical methods. A suitable name for it would be 'ultrastructural biology', which does not carry any particular implication as to the methods of investigation which should be employed. It is urged that this name should be used when the whole of this area of research is being referred to; the phrase 'molecular biology' could be retained, if desired, for that aspect of ultrastructural biology which deals with the chemical and physical study of the configuration of biologically important macromolecules.

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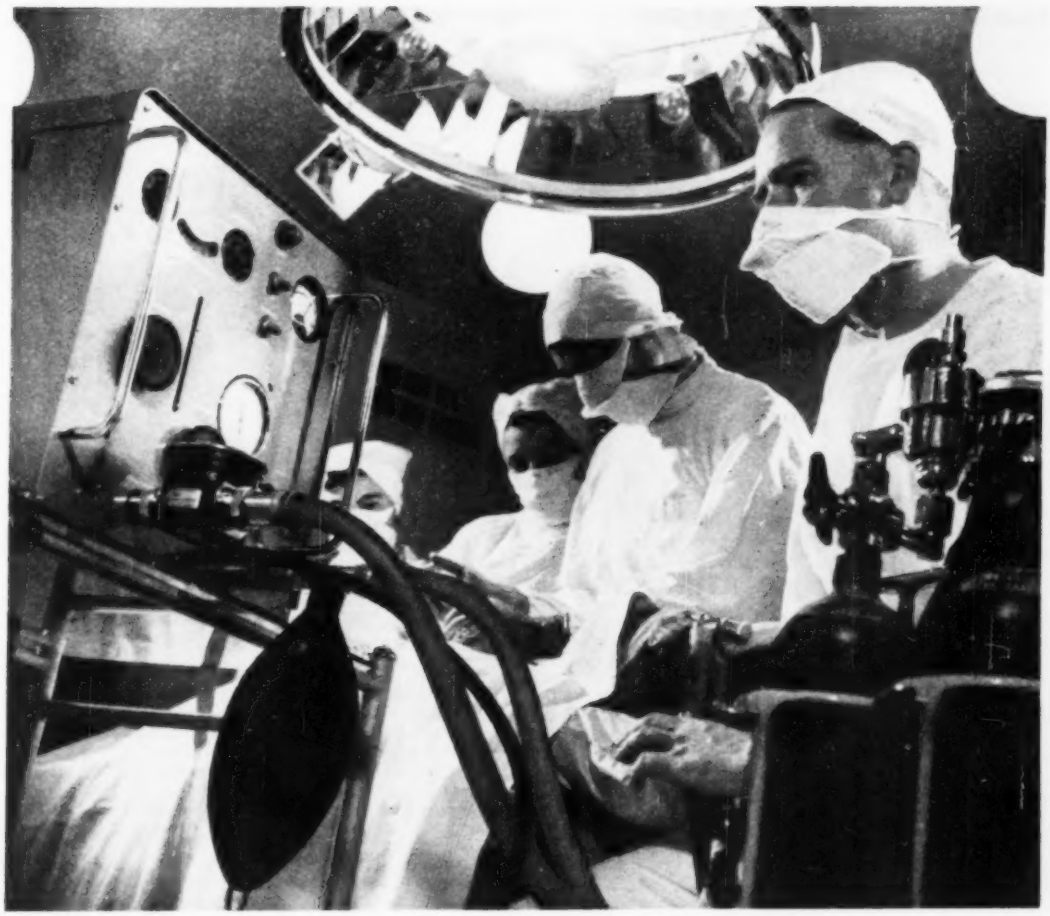
Influence of Uterine Position on the Response of the Mouse Embryo to the Teratogenic Effects of Hypervitaminosis-A

When a teratogenic agent is administered to a number of pregnant mammals of a single species under identical conditions of timing and dosage, its effect on the development of the young is by no means uniform. In any individual litter, all, none or a proportion only of the young may be deformed. Even when a highly inbred strain is used, variations in the response to the teratogenic agent may be attributable to differences in the maternal genotype. Such differences cannot, however, be held responsible for the situation in which individual members of a litter develop normally while others are deformed. The differences in response of litter mates must derive either from individual variations in genetic make-up or from environmental factors as yet unanalysed. The present investigation was carried out in order to determine the influence of a single environmental factor, namely, the site of implantation, on the susceptibility of the mouse embryo to the teratogenic effects of hypervitaminosis-A.

Forty female mice of the Strong *A* line were used in these experiments. Brother-sister matings only were used and the onset of pregnancy was determined by the presence of a vaginal plug, the day on which



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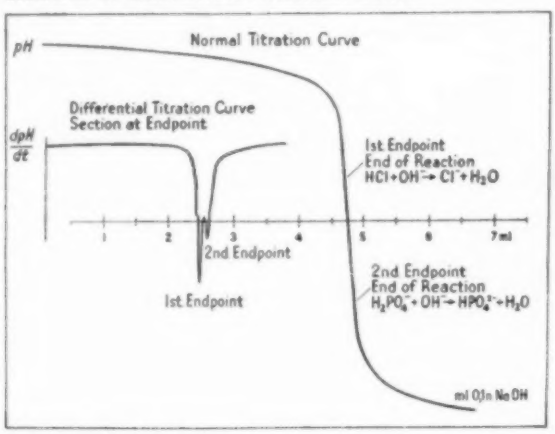

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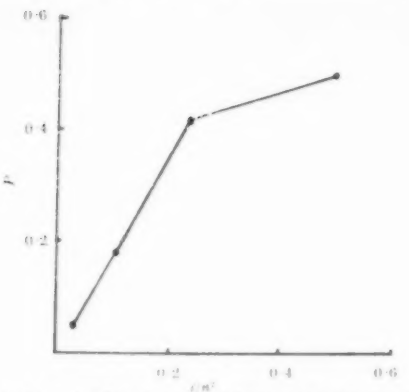

Fig. 1. *P* is probability of escaping deformity, *r* is ordinal position of a particular foetus measured from the vaginal side. *n* is total number of young in horn. (Both *r* and *n* vary from 1 to 8)

the plug was detected being taken as day 1 of the pregnancy. Each animal received 8,000 i.u. vitamin A acetate dissolved in 'Tween 80' (ref. 1) by subcutaneous injection on day 11 of pregnancy. On day 18 of pregnancy all the animals were killed. Each foetus was examined for the presence of cleft palate, and its position in the uterine horn was recorded.

Statistical analysis of the findings revealed two points of interest. First, foetuses situated at the ovarian end of the uterine horn were found to be less subject to cleft palate than those at the vaginal end (Fig. 1). Secondly, the probability of cleft palate increased as the number of young in the horn increased.

There are a number of possible explanations for these findings. Since the critical period for the production of a deformity by hypervitaminosis-A is quite sharply defined, the stage of development of an embryo might be expected to have a considerable influence on its response to the teratogenic agent. It is interesting to note, therefore, the observations of Mossman[2] that in the mammalian bicornuate uterus the embryos implanted nearest the oviduct are always more advanced than those at the vaginal end. The weight of the embryo and the degree of development of the placenta at the time when vitamin A is administered are both factors which may play a part in determining whether a malformation does or does not occur. McLaren and Michie[3] found that in the mouse the embryos in the top half of the uterine horn were heavier than those in the lower half at 18½ days *post coitum*.

McLaren and Michie[4] suggested that the blood supply at the site of implantation might be an important factor in determining the occurrence of runting. It is generally agreed that foetal weight in the mouse varies inversely with the number of young in the uterine horn. The more crowded the horn, the smaller are the individual foetuses. This may have some bearing on the finding of the present investigation that the highest incidence of cleft palate was in the more crowded horns. It is hoped that estimations which are now being made of the levels of vitamin A in the placenta and liver in normal and malformed litter mates will throw light on the connexion established by the present series of experiments between uterine position and the degree of susceptibility to hypervitaminosis-A.

Our thanks are due to Dr. P. Whittle of the Statistical Laboratory, Cambridge, for the statistical analysis and to Messrs. Vitamins, Ltd., for supplying the preparation of vitamin A. The work was supported by a grant from the Medical Research Council.

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Development of Eggs from a Hermaphroditic Sea Urchin

Reports of hermaphroditic sea urchins are not common. For *Arbacia punctulata* six examples are recorded over a period of twenty-five years by Harvey[1]. Since 1956, one report has been made[2].

In the course of work carried out in the summer of 1959, a hermaphroditic *A. punctulata* was found at Beaufort, North Carolina. On being subjected to an electric shock, two gonopores exuded sperm. Eggs were obtained from the remaining three pores. Each pore required individual stimulation in order for shedding to occur, and each gave viable gametes. The eggs were of normal size (80μ) for *Arbacia* collected in this area.

Since unfertilized eggs could be obtained, a study was made to compare the percentage fertilization and the rates of development of self-fertilized eggs and eggs fertilized by sperm from another male. As a control, additional eggs were reserved without sperm. All work was carried out at 23° C.

No indication of fertilization or cleavage was found in the controls. More than 85 per cent fertilization was obtained in both the self-fertilized and cross-fertilized egg batches. All stages of development up to the pluteus stage were directly comparable in both groups, and occurred at the normal rate for this temperature. No abnormal morphology was found.

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Disintegrated Spermatozoa from the Epididymis

In the course of an investigation of the response of spermatozoa in the epididymis of rabbits to the effects of artificial crytorchidism, it was noted that in the controls (that is, 12 normal epididymides on which no operative interference had been undertaken) a remarkable number of immotile decapitated spermatozoa was present in samples withdrawn from regions of the tubule proximal to the cauda epididymidis. Spermatozoa of this kind have been previously referred to as 'disintegrated spermatozoa'[1,2] and presumably indicate a process of degeneration.

In the present work the morphology of spermatozoa was examined in saline-diluted squash preparations under the phase-contrast microscope and in whole specimens. Quantitative estimates of particular characteristics were made from smears after staining with aqueous nigrosin eosin differential stain[3] in order that an estimate of the proportion of 'dead' (eosinophilic) spermatozoa also might be made. In view of this finding, spermatozoa from epididymides of some other species, namely the bull (6 specimens), the goat (2 specimens), the dog (4 specimens) and the cat (3 specimens) were also examined.

In all these cases a proportion of spermatozoa withdrawn from the vas deferens appeared to be degenerate; but it was possible to discern more evidence of disintegration in samples from the body of the epididymis. A variable percentage of dead and decapitated spermatozoa was observed in smears of samples taken from the head of the epididymis and from the initial segment[4]. These percentages were, however, generally lower than those in samples from the body of the epididymis. A relative paucity of degenerate forms from the tail of the epididymis in most specimens was typical. In bovine specimens the overall degree of disintegration of spermatozoa from all levels of the epididymis was comparatively small, but more eosinophilic spermatozoa were present in samples from the body of the epididymis than from any other level, which confirms an earlier report by Ortavant[5]. In both ruminants, differences between the incidence of disintegrated spermatozoa from the body of the epididymis and the cauda epididymidis were not conspicuous. Differences in mean values obtained for the incidence of decapitated spermatozoa from these two areas were, for the bull 8 per cent, and for the goat 16 per cent. In the dog, cat and rabbit, however, the contrast between samples from these two areas was striking, there being many more disintegrated spermatozoa from the body of the epididymis. Differences in mean values obtained between samples from the two parts in each of these species respectively were, 23, 66 and 49 per cent for decapitated spermatozoa, and 36, 63 and 61 per cent for eosinophilic spermatozoa. Despite considerable ranges of variation, differences between estimates from the two levels in these species were plainly significant.

From the observations it is not clear what proportion of disintegrated spermatozoa was due to artefact. The occurrence of disintegrated forms in whole samples where neither staining nor smearing was involved indicates that a number of spermatozoa were degenerate within the intact tubule of the epididymis, and thus some degree of wastage of spermatozoa in proximal regions of the epididymis is apparent. But in stained smears, it is conceivable that separation of the heads in several spermatozoa might have occurred *in vitro* and might thereby represent partly a variation in fragility of the cells at different levels of the tubule. In this connexion, it has been reported that decapitation of spermatozoa in ejaculated semen can be produced by certain methods adopted for the staining and clearing of smears[6]. Alternatively, differences in the viscosity of samples could interfere with the free movement of spermatozoa across the slide and give rise to an increased incidence of decapitated spermatozoa in smeared preparations. Subjectively, the milieu of spermatozoa from the tail of the epididymis appears to differ from that in the more proximal regions, especially in rabbits, and this might be related to possible differences in the effects of handling. Such variation in medium might also allow disintegrated spermatozoa to be obscured in smears of samples taken from the cauda epididymidis due to the effects of a specific distribution of degenerate forms. However, that the highest proportion of disintegrated spermatozoa was found throughout in samples from the body of the epididymis is of interest in that it suggests at least some characteristic quality of the tubular contents at this level. Experiments are now in progress to determine more precisely the inference to be drawn from this surprisingly regular pattern in the incidence of disintegrated spermatozoa from different areas of the epididymis.

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Tinsel-type Structure in Flagella of Zoospores associated with Cabbage Clubroot Tissue

PARTIALLY disintegrated clubbed roots of cabbage grown in the greenhouse in soil infested with *Plasmodiophora brassicae* Wor. were stored in a frozen condition for several weeks. They were then macerated in distilled water and much of the coarse debris was removed by filtration of the suspension through several layers of cheesecloth. This was followed by centrifugation at 4,000 r.p.m. for 15 min. and resuspension in sterile distilled water. Centrifugation was repeated three times and after the final one spores were suspended in the nutrient solution described by Macfarlane[1], or in autoclaved tap water in sterile 10 × 75 mm. culture tubes plugged with cotton. Tubes were incubated in the dark at 24° C. or at room temperature. Droplets were examined periodically under a Bausch and Lomb phase-contrast microscope for the presence of motile cells.

The occurrence of flagellated cells in such suspensions was unpredictable, but only one type of zoospore was encountered consistently in preparations from clubbed roots from three sources. Observations indicated that they were anteriorly biflagellate and markedly heterokont. The slightly recurved longer flagellum extended in front of the zoospore as it moved in droplets of the suspension. A much shorter flagellum was usually curved back along the body of the zoospore or extended forward at an angle with the longer flagellum. The zoospores rotated on a longitudinal axis as they moved forward freely and they varied from elongate to pyriform or globose in shape. Active zoospores measured 3 × 3μ–3·9 × 4·8μ. A pulsating vacuole was observed in most zoospores. Emergence of zoospores from germinating resting spores was not observed.

Permanent mounts were made using a modified Löffler's stain[2]. Droplets containing motile zoospores were placed on clean glass slides, and the zoospores were killed and fixed by exposure to the fumes of an aqueous solution of 2 per cent osmium tetroxide for 30 sec. The droplets were allowed to dry in a desiccator. Several drops of mordant were filtered on to

the dried preparation on the slide, which was heated gently for 45 sec. The mordant consisted of 20 per cent tannic acid, 10 parts; saturated aqueous ferrous sulphate solution, 5 parts; saturated alcoholic basic fuchsin solution, 1 part. The preparation was washed by dipping the slide in a large volume of distilled water. Several drops of stain were then added to the surface of the slide, which was heated gently for 1 min. The stain consisted of a 4 per cent solution of crystal violet in aniline water (2 parts aniline to 100 of water). Excess stain was removed by dipping the slide in several changes of distilled water. After drying in a desiccator, the preparation was mounted in Canada balsam with a No. 1 cover-slip.

Examination of stained preparations with a phase-contrast microscope indicated that the longer flagellum had a rather blunt end and was of the tinsel-type. The shorter flagellum was rounded at the end and bore no appendages. Stained zoospores measured 2·5–4 × 4μ. The long flagellum measured 6–8μ, while the short one measured approximately 2μ in length.

In the preparation of samples for the electron microscope, very small droplets of suspension containing active zoospores were placed on collodion-coated electron-microscope grids, exposed to fumes of 2 per cent osmium tetroxide for 30 sec., and allowed to dry in air. Grids were shadowed with uranium and examined with an *RCA*-type *EMU2* electron microscope. Examination of such preparations confirmed the nature of the flagella indicated by observations of the stained preparations. A photograph of a typical zoospore taken of an electron microscope preparation is illustrated in Fig. 1 (× *c.* 15,000).

The resting spore suspensions in which these zoospores were observed contained bacteria in varying concentrations, and occasionally flagellate cells other than those described were found. None of these possessed blunt-ended flagella. In one preparation, zoospores were observed which possessed a long flagellum trailing behind the zoospore as it moved. This flagellum was of the whiplash type, and the tailpiece was observed to vary in length as the zoospore advanced. Ellison[3] reported that the two heterokont flagella of primary zoospores of *P. brassicae* appeared blunt-ended without whiplashes or tinsels when stained by the modified Löffler technique. Kole[4] reported that secondary zoospores of *P. brassicae* moved with the longer flagellum trailing behind the zoospore. Zoospores stained by the modified Löffler method showed an apparent whiplash structure of the longer flagellum. The zoospores observed in preparations described here are believed to have developed by germination of resting spores of *P. brassicae*. It would appear from this work that the longer flagellum of the primary zoospore is typically of the tinsel type.

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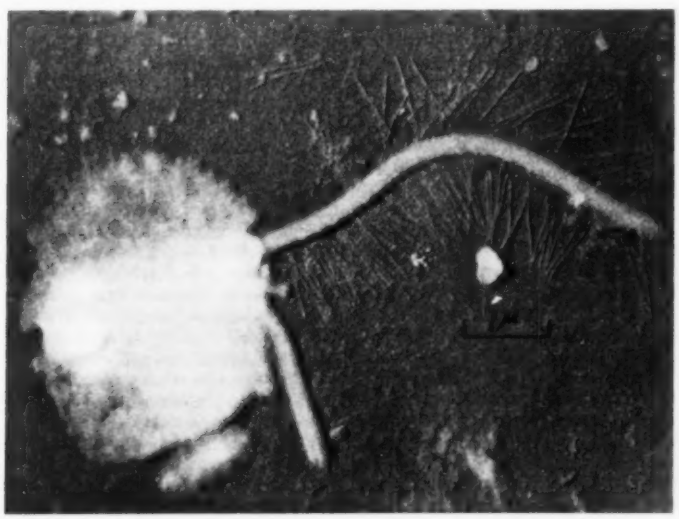

Fig. 1. Tinsel-type structure in flagella of zoospores associated with cabbage clubroot tissue

Substrate Specificity of the *Myrothecium* Ascorbic Acid Oxidase

The substrate specificity of the copper-containing ascorbic acid oxidase of higher plants was studied principally by Zilva *et al.*[1,2]. More recently, Mandels[3,4] discovered an ascorbic acid oxidase in spores of *Myrothecium verrucaria* (Alb. and Schw.) Ditm. ex Fr. which was termed 'atypical' chiefly because it differed from the classical copper-enzyme in substrate specificity and in its resistance to metal enzyme inhibitors. It did not oxidize D-glucoascorbate or D-arabonascorbate, and the latter inhibited oxidation of L-ascorbate. The mycelium of *M. verrucaria* also contains an atypical ascorbic acid oxidase, but with properties distinctly different from those of the spore-enzyme as well as of the copper-enzyme[5]. This communication describes the differences in substrate specificities.

The fungal oxidases were extracted from washed mycelial pellets or spores[6] by grinding in an ice bath with a Potter–Elvehjem homogenizer using 100-mesh glass and a *p*H 6·3, 0·01 *M* phosphate–0·005 *M* citrate buffer. This grinding was repeated twice following centrifugation at 0° C. for 10 min. at 1,000*g*. The pooled, cell-free extracts then were centrifuged at 0° C. for 30–40 min. at 20,000*g*. The resulting supernatant was diluted with buffer and stored frozen. Before use, the mycelial extract was acidified with 1 *N* hydrochloric acid to *p*H 4·5, the optimum for enzymatic activity. These fungal preparations showed no cytochrome oxidase or polyphenol oxidase activity. They had high catalase but little or no peroxidase activity. This evidence and the response to inhibitors of iron- and copper-enzymes[3,5] indicated that only ascorbic acid oxidase was involved in the activities measured. The copper-oxidase used was a crude cucumber juice prepared according to Johnson and Zilva[1].

Activity measurements were made at 30° C. in air by standard Warburg procedure at 2 ml. reaction volume, except with the rarer ascorbate analogues when 5-ml. flasks and 0·5-ml. volumes were used. The reaction buffers were 0·034 *M* phosphate–0·017 *M* citrate at *p*H 4·5 for the mycelial oxidase and *p*H 6·0 for the other two. The substrate concentrations used, 0·01–0·02 *M*, were sufficient for maximal velocity on the basis of previous reports[2,4] on the spore and cucumber enzymes and of work in this laboratory[5] on the mycelial enzyme. The concentration of the old samples of ascorbate analogues was based on amount of reduced form, determined by titration with 2,6-dichlorophenol indophenol or enzymatic oxidation with the copper-enzyme. Sufficient glutathione (30–60 μgm.) was added to all reaction mixtures to reduce autoxidation, and suitable autoxidation controls were run when necessary with certain analogues. Activities were based on initial reaction velocities since both fungal enzymes exhibited reaction inactivation[2,5], and rates of oxidation of some substrates also declined after 15–20 min. with the cucumber enzyme. It should be noted that the precision of the assay, particularly on the smaller scale and where autoxidation corrections were necessary, did not establish activity with analogues below perhaps 4–5 per cent of that with L-ascorbate. Thus 0 per cent in Table 1 may represent a low level of activity. The stoichiometry of analogue oxidation, like that of L-ascorbate, was one-half of oxygen uptake per mole of substrate.

Table 1. Substrate specificity of Ascorbic Acid Oxidase of spores and Mycelium of *M. verrucaria* and of Cucumber

Substrate	Relative enzyme activity (per cent)* Spore	Mycelium	Cucumber
L-Ascorbate (I)	100	100	100
6-Deoxy-L-ascorbate (II)	0	50	113
D-Xyloascorbate (III)	0	0	65
D-Araboascorbate (IV)	0	38–88	107
L-Araboascorbate (V)	0	0	34
L-Glucoascorbate (VI)	0	90	91
D-Glucoascorbate (VII)	0	0	19
Imino-D-glucoascorbate (VIII)	0	0	0
L-Rhamnoascorbate (IX)	0	98	97
L-Galactoascorbate (X)	0	100	97
D-Galactoascorbate (XI)	0	0	27
Imino-D-galactoascorbate (XII)	0	0	0
L-Guloascorbate (XIII)	0	0	35
Imino-L-guloascorbate (XIV)	0	0	0
D-Erythroascorbate (XV)	0	36–90	100
5,6-Diacetyl-L-ascorbate (XVI)	0	50	83
L-Erythroascorbate (XVII)	0	23	46
Hydroxytetronate (XVIII)	0	88	104
Reductate (XIX)	0	84	62
Methyl reductate (XX)	0	77	46
Reductone (XXI)	0	55	52
Dihydroxyfumarate (XXII)	0	0	0

* Average values except for ranges in case of mycelial enzyme with IV and XV, where activity varied significantly among enzyme preparations.

Of the 22 compounds tested only L-ascorbate (L-xyloascorbate) was oxidized at a significant rate by the spore enzyme (Table 1). Not only are Mandels's results for D-araboascorbate and D-glucoascorbate confirmed, but also it is evident that this enzyme has a very high degree of specificity. Any alteration in the molecule represented in this collection of analogues eliminated activity: (1) change in C-4 configuration, (2) change in C-5 configuration, (3) change in chain length, (4) substitution of hydrogen for hydroxyl on C-6, (5) modification or elimination of the ring, or (6) acetylation of the C-5 and C-6 hydroxyls. The inhibition of L-ascorbate oxidation by D-araboascorbate reported by Mandels[4] also was confirmed. Limited materials permitted only a single test of the other analogues, but it appeared that only 6-deoxy-L-ascorbate and L-galactoascorbate were inhibitory.

The mycelial enzyme was clearly less specific for the L-ascorbate structure (Table 1). Like the spore enzyme, it did not oxidize any of the 6- or 7-carbon analogues (III, V, VII, XI, XIII) with the opposite C-4 (ring) configuration to L-ascorbate. However, it did have some activity toward the 5-carbon analogue (XVII) with the opposite configuration. Among analogues with the same ring configuration, the 7-carbon compounds (VI, IX, X) were oxidized at about the same rate as L-ascorbate, while the rates with the 6-carbon compound (IV) were lower and variable. Substitution of a hydrogen for the hydroxyl group on C-6 of ascorbate (II) or acetylation of the C-5 and C-6 hydroxyls reduced activity to one-half. Removing the side chain (XVIII) or replacing the ring oxygen with a methylene group (XIX, XX) caused only a small reduction in activity. Even the 3-carbon analogue (XXI), with perhaps the simplest possible structure, was oxidized.

Presence of significant amounts of the spore-type enzyme in the mycelial extracts is unlikely, since several of the analogues were oxidized at about the same rate as L-ascorbate. Furthermore, low concentrations (0·0007 *M*) of D-araboascorbate had no effect on the rate of L-ascorbate oxidation by the mycelial enzyme but completely inhibited the spore enzyme.

The results in Table 1 with the cucumber extract were in close agreement with published values[1,2] for the same substrates and include data for a number of compounds apparently not tested before with the copper-oxidase (II, VIII, IX, XII, XIII, XIV, XVII XVIII XX). Analogues with the same ring configuration as L-ascorbate (II, IV, VI, IX, X) were oxidized at about the same rate. Presence of side chain or chain length (VI, XVIII), hydroxyl configuration at C-5 or C-6 (IV, VI, X), and lack of primary hydroxyl at C-6 (II) or C-7 (IX) had no apparent influence on activity. All analogues with the opposite ring configuration (III, V, VII, XI, XIII, XVII) were oxidized, but at a slower rate than L-ascorbate. None of the three iminoascorbates was oxidized, adding support to Dodds's conclusion[7] that a free carbonyl group is essential for activity. Compounds without the lactone ring (XIX, XX, XXI) were oxidized at lower rates, and the transdienol (XXII)[8] was inactive.

Nothing is known of the nature of the active sites of the two fungal enzymes, beyond the fact that these enzymes apparently are not typical copper-oxidases[2,3]. Their specificity patterns, however, give some indication of the substrate groups involved and of the stereochemistry of attachment. The spore oxidase would seem to require a multipoint attachment involving the hydroxyl groups on C-5 and C-6, the dienol group (since it is involved in electron transfer), and possibly the carbonyl group. Lack of a suitable derivative, such as imino-L-ascorbate, has prevented a test of this possibility, but a free carbonyl is required for the copper-enzyme. Such an attachment would be consistent with the absolute stereochemical specificity observed, which involves asymmetric centres at both C-4 and C-5. The highly specific nature of the binding site is also evident from the loss of activity on addition to C-6 of a methyl (IX) or hydroxymethyl group (VI), presumably by steric hindrance. With the mycelial oxidase, substrate structural effects were more quantitative than qualitative. A side chain was not required for attachment or activation, but its configuration (C-4) with respect to the ring was critical. With the unfavourable configuration, only the structure (XVII)

with the smallest side chain permitted any activity. Furthermore, with the favourable side chain configuration both the orientation of the C-5 hydroxyl and the presence of the C-6 hydroxyl influenced activity. Thus, the chain hydroxyls seem to participate in binding or to affect the orientation of the substrate at the reactive site.

A clear understanding of the difference in specificity between the spore and mycelial oxidases will require chemical characterization of the prosthetic group(s) and/or the reactive sites. This remarkable change in specificity of an enzyme between two morphological forms of the same organism provides an unusual opportunity to study the factors affecting enzyme specificity.

We are indebted to many people for samples of the rare analogues. This work was aided by a contract with the Office of Naval Research, Department of the Navy, *NR* 103-198, and by a Public Health Service research grant, *RG*-7354, from the Division of General Medical Sciences, U.S. Public Health Service.

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'Antibiotic Effects' in Plant Communities

FAILURE of *Araucaria cunninghamii* Sweet to regenerate in secondary rain-forest dominated by *Backhousia angustifolia* F. Muell. has been observed near Maryborough, Queensland. A plausible explanation of this observation appeared to lie in the widely discussed possibility[1] that a plant may interfere with the establishment of adjacent species in a natural community by releasing 'antibiotics', either from living roots or from decomposing surface debris.

On investigating this hypothesis it has been shown in laboratory tests that substrates, prepared from filter paper treated with *Backhousia* leaf oil, hinder the germination of cress and wheat. Further tests under similar conditions have revealed that dehydroangustione (in this case the major constituent of the oil[2]) hinders the germination of *Araucaria*, the effect varying with the concentration of dehydroangustione in the substrate. Thus, the number of radicles which emerged on substrates containing low (0·1–0·5 per cent by weight) and high (7 per cent) concentrations of dehydroangustione did not differ significantly from the control, but intermediate concentrations (1–5 per cent) caused a significant decrease ($P < 0.05$). Normal germination occurred and healthy seedlings developed on substrates containing 0–0·2 per cent dehydroangustione, but toxic symptoms appeared in all other treatments either during germination (1–7 per cent) or after the emergence of the whole seedling (0·5 per cent).

In an attempt to test the ecological implications of this finding, a small area of secondary rain-forest containing a high proportion of *Backhousia* was chosen. Four plots were then selected at random within this area and germination tests (using 200 *Araucaria* seeds per replicate) were set out on both the natural litter and on a litter enriched with freshly gathered *Backhousia* leaves which contained 5·4 per cent of dehydroangustione. A watering treatment was also included in the field test, which was carried out when germination normally occurs in this locality.

If the dehydroangustione present in *Backhousia* litter normally has an adverse effect on the germination of *Araucaria* in the field, it would be expected that any such 'antibiotic effect' would be enhanced under these conditions.

However, germination was virtually complete 37 days after sowing and statistical analysis of the results (Table 1), using the 'arc sine$\sqrt{p}$' transformation, revealed that there was a highly significant *increase* ($P < 0.001$) in germination on the litter containing fresh *Backhousia* leaves, compared with that on the natural litter. Moreover, germination on both watered treatments was significantly higher ($P < 0.01$) than on the corresponding unwatered treatments. At the conclusion of the experiment, both the unwatered, and watered, enriched litters were recovered from the field. Chemical analysis then revealed that these contained 1·6 and 2·2 per cent by weight, respectively, of dehydroangustione; that is, concentrations within the range found to hinder germination of *Araucaria* in laboratory tests.

This evidence does not eliminate the possibility that in certain circumstances germination of *Araucaria* may be inhibited by the dehydroangustione present in *Backhousia* litter. However, these circumstances have yet to be defined, and an investigation of the microclimatic factors affecting germination and early survival of seedlings in the field appears to be a more profitable line of inquiry.

Table 1

Substrate	Watering treatment	Percentage germination of *Araucaria* after 37 days: Replicate (*a*)	(*b*)	(*c*)	(*d*)	Mean
Normal litter	−	9	12	4	14	10*
	+	12	20	6	18	14
Enriched litter	−	20	18	10	19	17
	+	29	25	24	28	27

* In a nearby area the mean germination on an unwatered litter not derived from *Backhousia* was also 10 per cent.

In similar work, Went[3] suggested that the failure of some shrubs growing in the Southern Californian desert to harbour a population of certain annual species is due to the production of toxic substances by the shrubs concerned. This hypothesis was supported by Gray and Bonner[4], who isolated 3-acetyl-6-methoxybenzaldehyde from the leaves of one such shrub (*Encelia farinosa* Gray) and showed that this substance is a potent growth inhibitor in laboratory tests. However, Muller and Muller[5] criticized this interpretation of the field observations on the grounds that extracts of *Franseria dumosa* Gray, which does harbour these annuals, are even more toxic in laboratory tests than extracts of *Encelia*. These authors put forward several hypotheses to account for the failure of the toxic substances to reach an injurious concentration under natural conditions, and suggested that the distribution of these annual herbs may be

correlated with the superior water-holding capacity of the mound of organic debris accumulated by *Franseria*, but not by *Encelia*.

The apparently contradictory results of laboratory and field tests in this type of investigation may be due to the fact that all the toxic substances present in the substrate are available to the test species during the laboratory experiment when extracts or milled plant material are used. In our opinion such substrates are not comparable with plant litter, for it has been found in a replicated experiment that *Araucaria* seeds fail to germinate on substrates of milled *Backhousia* leaves enclosed in Petri dishes, but under the same conditions germination is not significantly reduced (although it occurs more slowly than the controls) on substrates of the same intact leaves containing 4·1 per cent of dehydroangustione.

In our opinion, the experiments outlined above emphasize the dangers of arguing, without further experimentation in the field, that the 'antibiotic effects' demonstrated in simple laboratory tests operate in natural plant communities.

Full details of this work will be published elsewhere.

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Sorbitol Metabolism in Apple Fruits

Sorbitol is a polyhydric alcohol found commonly in the Rosaceae: in apples it can constitute a large proportion of the dry matter. Little is known, however, of its metabolism in higher plants. Edson[1] referred to a sorbitol dehydrogenase in animals and micro-organisms and mentioned a similar occurrence in plants.

I have been able to show that, when an aqueous extract of an acetone powder preparation from the fruit cortex of apples (var. Miller's Seedling) was treated with sorbitol in the presence of boiled yeast extract, an acid-labile non-reducing sugar was formed. The sugar was probably sucrose and the normal yield was 1–2 mgm. from 10 mgm. sorbitol (at about 20° C.), with due allowance for the sucrose in the yeast extract.

These results were repeatable up to two months after preparation of the acetone powder. It then became inactive, and more detailed examination of the enzyme system involved in this reaction will be made when a further crop of fruit is available.

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BACTERIOLOGY

Association of a Steroid and a Pigment with a Diffusible Fruiting Factor in *Myxococcus virescens*

A diffusible factor inducing fruiting-body formation in Myxobacteria has been demonstrated by Lev[1]. In an attempt to identify further such a factor in *Myxococcus virescens*, an aqueous extract of fruiting-bodies was concentrated by drying from the frozen state.

Using a medium which would permit the swarming of vegetative cells, but not the formation of fruiting-bodies, under normal conditions, consisting of 1/100 concentration routine meat-extract agar, with 3 per cent non-nutrient agar and 0·001 per cent sodium chloride, four circular wells were made on a plate supporting a film of vegetative cells, and this was incubated for a further 7 days to obviate any effects of mechanical disturbance. A few drops of extract were placed in two of the wells, with distilled water in the two control wells. After 8 days incubation at 30° C., small fruiting-bodies could be discerned around the wells containing the extract.

Comparison of descending chromatograms of the two extracts showed the existence of a spot on the chromatogram of the fruiting-body extract which was absent from that of the vegetative cells. This spot was associated with the pigment of the fruiting-bodies; it reacted with Liebermann's reagent and is therefore presumed to contain a steroid.

A further experiment in which actual portions of the chromatogram paper were laid on the plate showed that fruiting-body formation was induced in the vicinity of the pigmented spot.

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Accumulation of Indole Compounds in Cultures of *Chromobacterium violaceum*

Substances yielding a typical indole test with *p*-dimethylaminobenzaldehyde (Ehrlich reagent) were recently observed in peptone broth cultures of gelatinous strains of *Chromobacterium violaceum*[1]. The nature and the origin of the substances giving the pink-red colour with Ehrlich reagent was of some interest because the species has been reported to lack the enzyme tryptophanase[2] and the genus as a whole is generally considered to be indole-negative[3], which implies inability to degrade tryptophan to indole.

Employing the Ehrlich reagent and technique described by Skerman[4], several cultures of gelatinous *Chromobacteria* were tested for indoles after growth for 5 days at 30° C. in several media. The results shown in Table 1 indicate that the largest accumulation of indoles occurred in 'Bacto' peptone broth containing glucose and incubated under aerobic conditions. 'Bacto' tryptone, which contains more tryptophan than peptone[5], did not allow indole accumulation. When 0·5 per cent (w/v) tryptophan was added to peptone broth no increase in the amount of Ehrlich-positive substances was observed. Synthetic glucose-ammonium salts medium with or without added yeast extract (0·02 per cent) invariably failed

Table 1. FORMATION OF INDOLE COMPOUNDS BY GELATINOUS STRAINS OF *Chromobacterium violaceum*

Medium	Relative intensity of the Ehrlich reaction 32 Shaken	32 Static	46 Shaken	46 Static	11 Shaken	11 Static
1 per cent peptone	+ + +	+	+ +	+ +	+ + +	+
1 per cent peptone, 1 per cent glucose	+ + + +	+ +	+ + + +	+ +	+ + + +	+ +
1 per cent peptone, 0·1 per cent tryptophan	+ + +	+	+ + +	+	+ + +	+
1 per cent tryptone	–	–	–	–	–	–
1 per cent tryptone, 1 per cent glucose	–	–	–	–	–	–

The cultures were incubated at 30° C. for 5 days in flasks mounted on a rotary shaker (shaken) or in deep tubes (static). Qualitative tests for indoles with Ehrlich reagent produced various intensities of pink colour (+ which were estimated visually. A negative sign indicates that no pink colour was observed.

to support the accumulation of indoles though good growth and pigmentation were usually observed. These results suggest that an abundant supply of amino-acids is a requisite for indole compound accumulation.

Chromatographic examination of glucose–peptone cultures, from which cells had been removed, revealed two substances giving a pink-red colour with Ehrlich reagent. Table 2 shows the R_F values of the substances in three solvent systems and the colour reactions produced when developed chromatograms were treated with reagents commonly used for the identification of indoles. Unless otherwise indicated, the methods and reagents employed were those described by Jepson[6].

Table 2. CHROMATOGRAPHY OF GLUCOSE–PEPTONE CULTURES OF STRAIN 32

	R_F values in A	B	C	Ultra-violet fluor-escence	Colour reactions with I	II	III	IV	V
Unknown 1	0·90	0·25	0·52	+	o—pk→b	v	r—bn	r	none
Unknown 2	0·72	0·19	0·25	+	pk→b	v	p	r	none
Indole-3-acetic acid	0·92	0·45	0·65	–	pk—p→vg	none	none	none	none
5 OH-indole acetic	0·82	0·25	0·34	+	pk→b	v	r—bn	r	none
Tryptophan	0·24	0·32	0·31	–	v—p	none	none	none	none
5 OH tryptophan	0·56	0·17	0·18	+	lt.b→b	v	r—bn	pk	none

Amounts of 10–20 μl. of cell-free culture fluid were spotted on Whatman No. 1 filter paper strips, and developed in the following solvents: *A*, butanol/acetic acid/water (4 : 1 : 5), *B*, *iso*propanol/ammonia (10 : 1), *C*, butanol/ethanol/water (4 : 1 : 1). Developed and dried strips were sprayed with I Ehrlich, II 1-nitroso-2-naphthol, III sulphanilic acid, IV anisidine, V 2,4-dinitrophenylhydrazine reagent. Colour reactions observed: orange (o), pink (pk), blue (b), purple (p), violet (v), grey (g), red (r), brown (bn).

Chromatography of the unknown substances with a number of known indole containing compounds indicated that the former were not indole, indole-3-aldehyde, indole-3-acetic acid, indole-3-propionic acid, indole-3-butyric acid, tryptophan, 5-hydroxytryptophan, 5-hydroxyindole-3-acetic acid or tryptamine.

Ehrlich reagent is not entirely specific for the indole nucleus, but because of the evidence obtained with other spray reagents, the known substances are believed to be indoles, more exactly hydroxyindoles. Udenfriend[7] has found that 1-nitroso-2-naphthol in the presence of weak acid and a trace of nitrite yields a violet colour with hydroxyindoles but not other indoles. The sulphanilic acid reagent described by Jepson[6] reacts not only with some phenolic acids but also with 4-, 5- or 6-hydroxyindole compounds. The latter also react strongly with anisidine reagent[8] to give an immediate, intense red-brown colour which is also true of the unknown indoles reported here. Phenolic acids do not apparently give the same immediate colour reactions with Ehrlich reagent under the conditions employed for indole detection. A negative test was obtained with 2,4-dinitrophenylhydrazine reagent which indicated the absence of keto- or aldehyde-groups.

Chromatography of peptone broth containing added tryptophan confirmed the observations described above, that is, it neither increased nor decreased the quantity of the two unknown indoles observed in peptone–glucose broth. Tryptophan did, however, give rise to several substances fluorescent in the ultra-violet which gave yellow colours with Ehrlich reagent. It seems clear that tryptophan is not the source of the unknown hydroxyindole compounds.

Chromobacterium species characteristically produce a purple pigment violacein, which has been shown to be a hydroxyindole-derivative[9]. DeMoss and Evans[10] have shown, using strain 553 (American Type Culture Collection), that pigmentation is dependent on L-tryptophan; in fact they have demonstrated that violacein was produced from the amino-acid by non-proliferating cells, when used as a sole carbon compound in the reaction mixture. These workers and others[11] have shown the hydroxylation of tryptophan to yield 5-hydroxytryptophan to be a major reaction for the amino-acid in this organism and probably is the source of the precursors of violacein.

In the present work L-tryptophan seemed to stimulate neither indole accumulation nor formation of pigment under the conditions used. These observations suggest that the gelatinous strains possess a pathway for synthesis of hydroxy indole compounds and pigment precursors not observed in non-gelatinous strain 553 or other strains dependent on tryptophan for pigment synthesis.

Identification of the hydroxyindoles and their relationship to pigment formation in the gelatinous strains is being investigated further.

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VIROLOGY

Rous Sarcoma Virus associated with Parthenogenesis in Turkey Eggs

SPONTANEOUSLY occurring parthenogenesis in unfertilized eggs of the domestic turkey, *Meleagris gallopavo* Linnaeus, was first noted[1] at the Agricultural Research Center, Beltsville, Maryland, in 1952. Unselected Beltsville Small White turkey hens, as virgins, produced eggs of which 16 per cent showed

parthenogenetic development following an incubation period of 9–10 days. This development consisted chiefly of a thickened sheet of unorganized and often multinucleated cells which covered varying areas of yolk surfaces. Usually, these cells did not attain the degree of organization necessary for blood formation and only on rare occasions were embryos noted. Through a system of selective breeding a strain of Beltsville Small White turkeys has been developed which regularly, as virgins, produce eggs of which 40 per cent can be expected to show some degree of parthenogenetic development. The greatest increase, by percentage, has been in the increased number of eggs showing a high degree of organized development. In 1959, embryos developed in 342 of the 2,926 eggs tested. This represented 11·7 per cent embryos, an increase amounting to 57 times that observed in 1952. Included were 12 poults which were helped from the shell and survived for some time (Olsen[2]).

In 1956, it was reported[3] that the incidence of parthenogenesis in turkey and chicken eggs could be significantly increased with an embryo-propagated live virus vaccine used for the control of the disease, fowl pox. Subsequent tests, at this Center, have confirmed these original observations. In 1958, Stolk[4] reported finding parthenogenesis in viviparous toothcarps following a local infection with the *Ichthyophonus* parasite, the phycomycete *Ichthyophonus hoferi* Plehn-Mulsow.

Work has since been extended to include other virus preparations, using unselected turkeys and observing the incidence of parthenogenesis in their eggs following inoculation. The present communication deals with the Rous sarcoma virus. Thirty unselected Beltsville Small White turkeys, housed in individual cages, were involved in the tests. Eighteen were inoculated subcutaneously in the wing webs with 0·1 gm. equivalent of partially purified Rous sarcoma virus (1 gm. equivalent represents the quantity of virus extracted from 1 gm. of tumour tissue). (I wish to thank Dr. Ray Bryan, U.S. Public Health Service, Bethesda, for the virus extract from chicken tumours.) The other 12 birds, full sisters of those of the treated group, served as uninoculated controls. Eggs from both groups of females were incubated for 10 days before being examined macroscopically for indications of parthenogenetic development. The percentage incidence of parthenogenesis observed over successive 6-day intervals in eggs laid by treated and control birds is presented in Table 1. During the 90-day test period following inoculation, 714 eggs were produced by the 18 treated turkeys, 527 by the 12 control birds. Of all eggs produced by the treated birds, 25·4 per cent showed parthenogenetic development, while 15·9 per cent of the eggs from the control group were so classified. The difference, when tested by the analysis of variance using weighted squares of means, was found to be highly significant at the 1 per cent level. (Thanks are due to Mrs. R. R. Taylor of this Center for this analysis of data.)

Table 1. Percentage Incidence of Parthenogenetic Development observed in Eggs of Beltsville Small White Turkeys following Inoculation with Rous Sarcoma Virus

No. of days following inoculation	Treated birds		Controls (untreated)	
	Eggs tested	Percentage parthenogenesis	Eggs tested	Percentage parthenogenesis
22–27	72	11·1	55	15·1
28–33	69	22·6	52	13·5
34–39	59	20·3	42	16·7
40–45	65	32·3	46	15·2
46–51	65	26·1	50	18·0
52–57	67	35·8	49	10·2
58–63	64	28·6	46	15·2
64–69	74	21·6	50	16·0
70–75	53	15·1	46	26·1
76–81	56	33·9	42	16·7
82–90	70	28·6	51	15·7
Totals and averages	714	25·4	527	15·9

The results presented clearly indicate that Rous sarcoma virus tends to increase the incidence of macroscopically observable development in unfertilized turkey eggs.

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Bromegrass Mosaic Virus : a Virus containing an Unusually Small Ribonucleic Acid

Chemical and physical properties of viruses are being investigated in several laboratories in order to obtain structural information which relates to questions of virus synthesis and genetics. Bromegrass mosaic virus, a spherical plant virus, may qualify as a useful test object for such investigations. Data reported here show that this virus is particularly small, and furthermore that the molecular weight of its ribonucleic acid is much smaller than that of any other known virus.

The sedimentation coefficient of bromegrass mosaic virus was measured in a Spinco model *E* analytical ultracentrifuge at 23·3° C. in 0·1 ionic strength buffer (sodium phosphates + sodium chloride, *p*H 6·0) at 35,600 r.p.m. Runs were made at concentrations of 0·12, 0·25, 0·37, 0·49 and 0·99 per cent, and the resulting plot of sedimentation coefficient versus concentration was extrapolated to infinite dilution. The extrapolated value, 92·0 *S*, was corrected to the value expected in water at 20° C. Thus, $s^{\circ}_{20,w} = 86{\cdot}2\ S$. Diffusion measurements were carried out in a quartz Tiselius cell in a Spinco model *H* electrophoresis-diffusion apparatus at 2° C. in the same buffer as above. The virus concentration was 0·42 per cent. Apparent diffusion coefficients were calculated according to the method of Longsworth[1] from five photographs of the Rayleigh fringe patterns taken at selected time-intervals. From these results the diffusion coefficient was calculated to be $8{\cdot}62 \times 10^{-8}$ cm.²/sec., and this value was corrected to that expected in water at 20° C. Thus, $D_{20,w} = 1{\cdot}55 \times 10^{-7}$ cm.²/sec. From the sedimentation and diffusion coefficients at a virus concentration of 0·42 per cent and an assumed partial specific volume of 0·71 ml./gm., the molecular weight of bromegrass mosaic virus was calculated to be $4{\cdot}6 \times 10^{6}$. To our knowledge this is the lowest molecular weight reported for any virus.

The nucleic acid content of bromegrass mosaic virus was determined by means of the optical method of Englander and Epstein[2], in which the nucleo-

protein concentration is determined by a measurement of the refractive index increment of a solution, and the nucleic acid concentration is determined by a corresponding measurement of the optical density at 260 mμ. The measured optical density of the virus solution was corrected for the effect of light scattering and for absorption due to protein. (The extinction coefficient for ribonucleic acid at 260 mμ was taken to be 22·3 cm.²/mgm., the specific refractive increment to be $1{\cdot}8 \times 10^{-4}$ ml./mgm. and the protein extinction coefficient to be 0·5 cm.²/mgm.) The ribonucleic acid content was found to be 21 per cent. By analogy with the reported characteristics of the ribonucleic acid of other small viruses, it is reasonable to assume that bromegrass mosaic virus ribonucleic acid is all in one piece. Its molecular weight is then 1×10^6. This is a lower molecular weight than that of any other known genetically functional nucleic acid[3,4].

Experiments were undertaken to show that the 4·6 million molecular weight particle described above is indeed the infectious virus. These experiments consisted of infectivity and particle assays of fractions obtained by density gradient centrifugation as follows: A 10–40 per cent w/v sucrose density gradient (*p*H 6·5 in 0·1 ionic strength phosphate buffer) was prepared according to the method of Bock and Ling[5]. A 0·25-ml. sample of a 0·53 per cent solution of bromegrass mosaic virus was layered on top of the gradient solution and this was centrifuged at 25,000 r.p.m. for 3 hr. in an *SW*-25 swinging bucket rotor of a Spinco model *L* centrifuge. A single, opaloscent band corresponding to the 4·6 million molecular weight particle was observed in the centrifuge tube at the conclusion of the run. The tube bottom was punctured with a hypodermic needle, and 1-ml. fractions were collected and tested for infectivity on the local lesion host *Chenopodium hybridum*[6]. Fractions 1–5, 6–10, 11–15 and 25–29 were pooled. 0·1-ml. samples of the pooled fractions and the remaining fractions 16–24 were brushed on to alternate right and left half-leaves after dusting them with 'Carborundum'. The opposite half-leaves were brushed with a standard bromegrass mosaic virus solution. Adjustment of the local lesion count for difference in susceptibility of leaves was made. Results are shown in Fig. 1. It may be seen that the maximum infectivity coincides with the maximum particle concentration as measured by optical density, and moreover the entire set of infectivity and optical density values superpose very well. Since the separation into fractions was essentially on the basis of sedimentation velocity it may be concluded that the infectious agent sediments at the same rate as does the 4·6 million molecular weight particle.

A similar experiment was performed with a 35–38 per cent w/w rubidium chloride density gradient (same buffer as above). Bacteriophage φ*X*174 top and bottom components the particle densities of which in rubidium chloride are 1·32 and 1·40, respectively, were added to the virus solution as markers in order to establish the resolving power of the procedure. The density of the 4·6 million molecular weight particle is intermediate to these two values. After 12 hr. of centrifugation three bands were observed in the density-gradient tube. Infectivity analysis (carried out by Robert K. Fujimura) of appropriate fractions (by plaque counts on *E. coli C*) verified that the optical density in the first band was due to φ*X*174 bottom component. The second band contained the 4·6 million molecular weight particle, identified by its intermediate density and distinctive ultra-violet absorption spectrum. The third band contained φ*X*174 top component, verified by its distinctive ultra-violet absorption spectrum. Infectivity on *Chenopodium hybridum* showed that only the fractions corresponding to the second band contained infectious bromegrass mosaic virus. Since the particles were separated in this experiment according to their densities, it may be concluded that the infectious agent and the 4·6 million molecular weight particle have the same density.

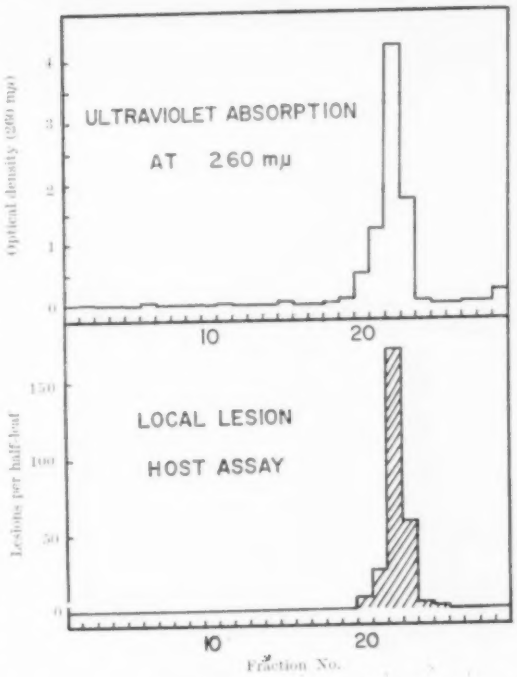

Fig. 1. Ultra-violet absorption at 260 mμ and infectivity assays of fractions obtained from sucrose density gradient (10–40 per cent w/v) centrifugation of bromegrass mosaic virus

Thus, we conclude on the basis of velocity and equilibrium sedimentation experiments that the particle, the characteristics of which we have described, is indeed the infectious virus.

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GENETICS

Genetic Studies with *Klebsiella pneumoniae*

Genetic recombination following conjugation and phage transduction has been reported within and between several groups of enteric bacteria. It was decided, therefore, to look for evidence of genetic exchange in *Klebsiella pneumoniae*, and between this organism and others of the Enterobacteriaceae.

By means of the penicillin selection technique stable auxotrophic mutants were obtained in 17 *K. pneumoniae* strains. Strain *NCTC* 8821 readily yielded unstable auxotrophs with high rates of reversion to prototrophy, and may perhaps carry a mutation factor like that described by Jyssum[1] in strains of *Escherichia coli*. Strain 8821 did not, however, have a high rate of spontaneous mutation to streptomycin indifference.

Attempted transductions, employing 11 *Klebsiella* phages isolated from pooled sewage, gave only one suggestive result: a cysteine auxotroph of strain *NCTC* 8172 was apparently transduced to prototrophy at low frequency. The possibility that the rare prototrophs resulted, however, from transformations by deoxyribonucleic acid present in the crude phage lysate has not been excluded.

Mixed cultivation experiments were carried out according to the method of Miyake and Demerec[2]. These failed to yield any evidence of genetic recombination, occurring at the rate of at least 1 recombinant per 10^7 cells plated, between the different *K. pneumoniae* strains, between *K. pneumoniae* and *E. coli K-12*, HfrH, or *K. pneumoniae* and strain *NCTC 8706* of *Chromobacterium prodigiosum*.

An interesting syntrophic interaction was found between an unclassified auxotroph of *C. prodigiosum* 8706 and some *K. pneumoniae* auxotrophs. When well-washed cells of the *C. prodigiosum* mutant were spread on minimal *A* agar[3] plates, then incubated at 30°, few prototrophic revertant colonies appeared. Numerous reversions arose, however, if this auxotroph were incubated on minimal medium supplemented with a small amount of broth, or if washed mixtures of the *C. prodigiosum* and *K. pneumoniae* auxotrophs were incubated on minimal medium.

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PSYCHOLOGY

Effects of Mental Activity on Vestibular Nystagmus and the Electroencephalogram

Mowrer[1] noted differences in head nystagmus elicited by rotation under conditions of 'excitement' and 'rest' in pigeons. Wendt[2] has indicated that "inward-directed revery states" reduce nystagmus and that the subject must "maintain an environment-directed orientation" to avoid replacement of nystagmus by wandering, autogonous eye movements. The present work was undertaken to ascertain how nystagmus, produced by a standard vestibular stimulus, is modified by different sets of instructions and, further, to determine the effects of these conditions on the electroencephalogram.

Subjects were seated in a light-proof room in the centre of a rotary apparatus, described in detail by Guedry and Kalter[3]. An adjustable biteboard held the subject's head in a standard position and a telegraph key, mounted on the right arm rest, was used as a signalling device. An Offner type *T* encephalogram, secured to the table, permitted the simultaneous recording of encephalograph (from a pair of parieto-occipital leads), horizontal nystagmus, subject key presses and a marker which denoted the periods of acceleration and deceleration. Subjects met three criteria: (*a*) no history of labyrinthine disorder; (*b*) production of alpha activity when relaxed with eyes closed; (*c*) quantifiable nystagmus. Of the seven candidates tested, only one was eliminated (due to a lack of alpha production). All subjects had previous turntable experience.

Three tasks were selected: (1) key pressing-signals made by the subject to indicate onset, cessation, and each experience of 90° of subjective rotation; (2) mental arithmetic-subjects were instructed to divide 80 and each successive quotient by 5, ignoring the external environment, and proceeding as rapidly and accurately as possible (answers were recorded); (3) reverie-subjects were told to ignore the environment and simply to relax and daydream without directing their thoughts in any way. The tasks were given in the sequence above and repeated in the same order. The six trials were conducted successively on a single day.

All trials were conducted in the dark with eyes open, head upright, and rotation in a clockwise direction. Each trial began with a 30-second period of rotation at 1 r.p.m. followed by 14·9 sec. of 4·5°/sec.² acceleration to a constant velocity of 12·2 r.p.m. This speed was held for 2 min. before the subject was decelerated at 4·5°/sec.² to 1 r.p.m. After 1 min. of rotation at this velocity, the turntable was stopped and the trial ended. Prior to each trial, subjects alternately fixated markers for calibration purposes. They were located so as to provide horizontal eye sweeps of ±20° to either side of a central fixation point.

The slow-phase amplitude of the nystagmus was measured in centimetres, summed for the 14·9-sec. periods of acceleration and deceleration respectively, and converted to total degrees of slow-phase eye movement. The measurements made were the vertical distances from the peak of each nystagmic beat to the slow-phase base-line of that beat. Durations were calculated from the start of stimulation to the point where the primary nystagmus ended. The data from the encephalograph were tabulated by the 'seconds of alpha' technique, and the scoring covered only the periods of acceleration and deceleration.

Much less nystagmus was present during the daydreaming than during the key pressing or arithmetic trials (Fig. 1). The scored observations were subjected to separate analyses of variance and *t* tests. For acceleration, the conditions did not yield significantly different results. The deceleration results yielded the following significant relationships: (*a*) smaller total

Fig. 1. Nystagmus and electroencephalograph tracings for subject 4 showing trials 2, 3 and 4, with the subject-tasks of mental arithmetic, reverie and key pressing, respectively (paper speed, 25 mm./sec.). The heavy vertical bars demarcate the period of 4·5°/sec.² deceleration of 14·9 sec. duration. The calibrations to the right of each eye movement record indicate 20° eye excursions and the 100 μV. calibration refers to all electroencephalograph tracings. The amplifier time-constant for eye movements was 1·4 sec. Note that the contiguous task is a powerful variable in determining the qualitative as well as the quantitative aspects of nystagmus (during the period of deceleration, *MA* = 363, *REV* = 151 and *KP* = 380 degrees of slow-phase eye movement). The alpha rhythm as well as the nystagmic output are reduced during the reverie condition

degrees of nystagmic output during reverie than key pressing or mental arithmetic (0·03 and 0·001 levels respectively); (*b*) shorter durations of nystagmus for reverie conditions than for key pressing or mental arithmetic (0·01 levels); (*c*) more alpha during mental arithmetic than key pressing or reverie (0·02 levels).

The results demonstrate that nystagmus can vary with type of mental activity, thereby confirming Wendt's claim. However, it does not seem necessary that the subject be 'environment-directed' (as in the key pressing condition) in order to maintain nystagmus. Subjects frequently reported a lack of awareness of acceleration, and more often of deceleration, while doing arithmetic. Although this was also true for reverie states, the nystagmic output remained high during the mental work. Thus, general alertness would appear to be the important factor in maintaining nystagmus.

The failure to obtain significant differences from the acceleration results seems due to a combination of factors. One of these was an alerting 'end effect'. Two of the three subjects who knew the number of trials to be conducted produced more nystagmus during the last acceleration (reverie) than during any preceding trial; the third exceeded all but one previous trial. That such knowledge can 'alert' the subjects and increase the nystagmic output has been noted elsewhere[4]. The remaining factors were deliberately included in the design in order to determine the power of the mental set variable. They included: (*a*) the rapid change from mental activity to reverie required of the subjects; (*b*) the relatively brief period (30 sec.) between instructing the subjects to daydream and initiating the acceleration stimulus. Further work in progress demonstrates clearly that nystagmus declines significantly while subjects are daydreaming during both acceleration and deceleration when more favourable conditions are employed for the establishment of mental 'sets'[5].

It was anticipated that alpha activity would be more evident during reverie and would correlate negatively with nystagmic output. However, more alpha was present during the arithmetic task for the subjects studied here. Although this specific encephalograph finding may be peculiar to the particular group of subjects employed, or to the experimental conditions, the evidence from this investigation and others in progress indicates that: (*a*) nystagmus is markedly sensitive to instructions regarding mental activity while the pattern of the encephalograph is not; and (*b*) vestibular stimulation in combination with various attention-demanding tasks does not block alpha production which is going on[5]. It is frequently stated that various types of sensory input as well as mental activity will block alpha, but other reports note that such is not always the case[6–8].

That gross differences in nystagmic output depended on the mental 'set' of the subject (one subject gave no nystagmus at all during a day-dreaming session) and that this variable is so sensitive as to be manipulated within seconds by simple instructions should be of interest to clinicians seeking to evaluate vestibular dysfunction. It may also explain the lack of a 'constant reaction' and the 'temporary reduction in response' noted for some of the subjects recently studied by Suzuki and Totsuka[9].

Our findings suggest that: (*a*) alertness, whether 'environment-directed' or not, is a highly significant variable in the maintenance of a vigorous nystagmic response; (*b*) subjects should be given objective tasks as a control feature during vestibular experimentation; (*c*) under certain conditions, eye movements may give better indications of a subject's state of alertness than do encephalograph patterns.

We gratefully acknowledge the expert technical assistance of Stanton Warburton.

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The 'Psychological Refractory Phase' and Ageing

When, in psychological experiments, the subject is required to respond as rapidly as possible to a signal closely following another, an increase in response latency is observed[1]. It has become customary to refer to this aspect of high-speed sequential performance as the 'psychological refractory period' in analogy with changes in threshold of neurones, following the propagation of impulses, where the recovery is divisible into an 'absolute' and a 'relative'

phase. Welford[1] has argued that the slowing of response to the second of two successive signals is due not to a temporary reduction of central sensitivity, but to the feature that "no two central organizing times can overlap". His view is derived from that of Hick[2], who postulated that in continuous performance the brain is occupied not only in dealing with a particular signal but also with the monitoring of a response to it.

The experiment briefly reported here differs from previous work on psychological refractoriness in three ways. First, the subject was required to deal simultaneously with two streams of signals, one regular, highly probable input (for which a response was made with the non-preferred hand), and the other irregular, with high temporal uncertainty (for which a response was executed with the preferred hand). Secondly, in contrast with most previous experiments, the 'second' signal, when pairs of signals were presented, always occurred after the response to the 'first' signal had been initiated. Intervals of 50, 150, 300 and 600 msec. between the beginning of a regular response and the onset of an irregular signal were employed. Thirdly, the regular signals were presented at two different rates; (*a*) at one every 2 sec.; (*b*) in another session, at one every 4½ sec. 34 subjects, ranging from 20 to 80 years of age, and divided into three age-groups, took part in the experiment. Approximately half the subjects in each group began with the faster rate.

The average response times to the irregular input for the three age-groups are shown in Table 1. (Although omitted to save space, the response latencies to regular signals also decreased with increased rate.)

Table 1. Mean Response Lag (msec.) to the Irregular Signal

Age group	Interval (R1–S2)			
	50	150	300	600
	At 'fast' rate of the regular sub-task			
20–39 (11 subjects)	319	300	298	307
40–59 (12 subjects)	320	306	292	310
60–80 (11 subjects)	376	356	339	345
	At 'slow' rate of the regular sub-task			
20–39 (11 subjects)	329	295	299	317
40–59 (12 subjects)	358	302	299	323
60–80 (11 subjects)	419	374	364	374

Analysis of these results suggests that the period over which reaction times were lengthened is less than 150 msec. but more than 50 msec. for all subjects at both rates ($P < 0{\cdot}05$—$P < 0{\cdot}001$), and greater than 150 msec. but less than 300 msec. for the middle-aged and older subjects when the regular signals appeared at the faster rate ($P < 0{\cdot}02$). However, the magnitude of the delays is lower than would be predicted on Welford's theory, and it is doubtful if we can, without further qualifications, refer to the range of intervals over which response times increase as the 'refractory period', in the sense of 'dead time'. If the occupation of the central mechanisms by previous signals, or by feedback, prevents the commencement of the processes concerned with the subsequent signal, the response lag should equal the difference between the duration of the 'refractory period' and the time elapsing before the second signal is delivered, that is, it will give a curve with negative slope of 45°. The slopes obtained in the present experiment are considerably less than this in all cases, suggesting that the delays observed may be due not to the start of the processing of the irregular input being prevented, but to the decision mechanism operating less quickly for a short time after it had again become free to deal with signals. Such an interpretation, if correct, implies that the analogy with physiological refractoriness is more exact than hitherto assumed. Possibly it is important to distinguish between an 'absolute phase', in which signal analysis is held up until the central processes already begun have ended, and a 'relative phase' during which the speed of decision is lowered. The duration of both phases is likely to be a function of signal probability, and it is in suggesting this relationship that our results differ from previous ones. Certainly, in our investigation the distinction is of interest, because although for the older subjects an increase in the frequency of regular signals leads to a lengthening of the 'relative refractory period', what can be inferred about the duration of the 'absolute phase' from the slope points to a decrease (mean difference between the two rates × age: at 50 msec. $P < 0{\cdot}02$, at 150 msec. $P < 0{\cdot}05$, at 300 msec. $P > 0{\cdot}05$, at 600 msec. $P < 0{\cdot}02$; mean difference in slopes 50–150 msec. intervals at the two rates: $P > 0{\cdot}05$ for young subjects, $P < 0{\cdot}001$ for both the middle-aged and old groups).

These observations suggest the possibility that amplification of a routine activity may serve, in the case of the older adults, to improve selective responsiveness of central mechanisms to a high-information signal. If such an amplification causes the level of 'vigilance'[3] or 'arousal'[4] to be increased, perhaps via the reticular formation, it is conceivable that decision processes are thereby enhanced. There are, of course, many factors determining the level of activity in the reticular system[5]; but there is direct neurophysiological evidence that one of them may be the rate of sensory inflow[6]. In addition to our observations, the more recent results of comparative experiments[7] would seem to support such an interpretation.

In an attempt to explore these theoretical possibilities, we are now engaged in examining older adults, employing different sensory channels for each stream of signals. Preliminary results suggest that response latencies and refractory delays to visual signals tend to decrease still further with an auditory sub-task. As a further step we are attempting to develop a suitable technique for observing refractoriness in continuous performance, when the size of the ensemble of possible signals varies.

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FORTHCOMING EVENTS

Monday, April 10

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Applications of Electrical Phenomena at Liquid Helium Temperatures" opened by Dr. R. E. Hayes.

SOCIETY OF ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. A. E. Canham: "The Use of Electric Light and Heat in Horticulture".

ROYAL AERONAUTICAL SOCIETY, HISTORICAL GROUP (at 4 Hamilton Place, London, W.1), at 7 p.m.—Discussion Evening—"A Quiz on Little Known Early Aircraft".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. H. W. Tilman: "Voyage to Iles Crozet and Kerguelen".

Monday, April 10—Saturday, April 15

FIRST INTERNATIONAL CONGRESS ON "METALLIC CORROSION" (at Imperial College, Prince Consort Road, London, S.W.7).

Tuesday, April 11

BRITISH OCCUPATIONAL HYGIENE SOCIETY (at Birkbeck College, Malet Street, London, W.C.1)—Conference on "Training in Occupational Hygiene".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND ELECTRONICS SECTIONS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. Woods: "Precision Instruments for Co-axial Line Measurements up to 4 Gc./s.". (Supported by a paper by Mr. D. Woods entitled "The Concept of Equivalent Source E.M.F. and Equivalent Available Power in Signal-Generator Calibration".)

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Prof. M. W. Thring: "Direct Electrical Power Generation by Thermal and Chemical Means".

Tuesday, April 11—Thursday, April 13

ROYAL PHOTOGRAPHIC SOCIETY SCIENTIFIC AND TECHNICAL GROUP; ROYAL GEOGRAPHICAL SOCIETY; and the BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7)—Symposium on "Photography on Expeditions".

Wednesday, April 12

INSTITUTE OF PHYSICS AND PHYSICAL SOCIETY, LOW TEMPERATURE GROUP (at 47 Belgrave Square, London, S.W.1), at 11 a.m.—Meeting on "Techniques for Generating Very High D.C. Magnetic Fields".

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. L. R. Raymond: "The Petrology of the Permian Rocks of North-east Yorkshire and South-east Durham—The Lower Magnesian Limestone"; Dr. D. K. Bailey: "Intrusive Limestones in the Keshya and Mkwisi Valleys, Northern Rhodesia".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Dr. R. N. Curnow: "Optimal Programmes for Varietal Selection".

BRITISH INSTITUTION OF RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. D. E. Mullinger: "Vibration Analysis and Testing".

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Teaching Physics to Engineers", opened by Dr. J. Topping.

SOCIETY FOR ANALYTICAL CHEMISTRY (joint meeting with the OIL AND COLOUR CHEMISTS' ASSOCIATION, at the Wellcome Building, Euston Road, London, N.W.1), at 7 p.m.—Scientific Papers.

WOMEN'S ENGINEERING SOCIETY (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7 p.m.—Miss Sabey: "Road Research".

ROYAL AERONAUTICAL SOCIETY, GRADUATES' AND STUDENTS' SECTION (at 4 Hamilton Place, London, W.1), at 7.30 p.m.—Mr. B. S. Shenstone: "Man-Powered Flight—Progress to Date".

Thursday, April 13

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Miss Mary F. Hay, Mr. H. R. Lindner and Dr. T. Mann, F.R.S.: "Morphology of Bull Testes and Seminal Vesicles in Relation to Testicular Androgens"; Mr. T. W. Glenister: "Organ Culture as a New Method for Studying the Implantation of Mammalian Blastocysts".

INSTITUTION OF MECHANICAL ENGINEERS; INSTITUTION OF CIVIL ENGINEERS; and the INSTITUTION OF ELECTRICAL ENGINEERS (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—H.R.H. The Prince Philip, Duke of Edinburgh, K.G.: "Resources, Human and Material, of the Commonwealth" (Graham Clark Lecture, 1961).

Friday, April 14

ASSOCIATION OF APPLIED BIOLOGISTS (in the Metallurgy Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 10.15 a.m.—Annual General Meeting.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

GRADE B ASSISTANT TO TEACH BIOLOGY to G.C.E. Advanced level and to Pre-nursing classes with some General Science; and a GRADE B ASSISTANT TO TEACH PHYSICS to G.C.E. Advanced level and to O.N.C. classes—The Principal, Peterborough Technical College, Easterfield Road, Peterborough (April 15).

SENIOR RESEARCH SCIENTIST (of high academic standing in biochemistry, and preferably with a medical qualification), to take charge of a new laboratory unit and undertake research into the biochemical aspects of cancer—The Secretary, Marie Curie Memorial Foundation, 124 Sloane Street, London, S.W.1 (April 15).

SENIOR LECTURER IN MATHEMATICS—The Principal, Nottingham Training College, Clifton, Nottingham (April 17).

CHAIR OF ZOOLOGY tenable in the Durham Division of the University—The Registrar, University Office, 46 North Bailey, Durham (April 22).

LECTURER (preferably with qualifications in historical geography with special reference to Scotland) IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, The University, Aberdeen (April 22).

RESEARCH FELLOW IN MATHEMATICS—The Secretary, Royal College of Science and Technology, Glasgow (April 22).

SENIOR LECTURER (prepared to initiate research in some branch of pure or applied mathematics) IN MATHEMATICS—The Secretary, Royal College of Science and Technology, Glasgow (April 22).

STATISTICIAN, Scientific Officer (with a good honours degree in mathematics or science and a knowledge of statistical techniques as applied to agriculture or biology) at the Agricultural Research Council's Unit of Statistics (University of Aberdeen)—The Secretary, Agricultural Research Council, Cunard Building, 15 Regent Street, London, S.W.1 (April 22).

LECTURER IN THE DEPARTMENT OF PHYSICS—The Registrar, University College of North Wales, Bangor, North Wales (April 24).

LECTURER IN CYTOLOGY in the Departments of Botany and Zoology in the Faculty of Science—The Registrar, The University, Manchester 13 (April 25).

ASSISTANT LECTURER (Grade B) IN GEOGRAPHY—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (April 29).

ASSISTANT MYCOLOGIST (preferably male, with M.Sc. or B.Sc. degree in botany, forestry or agriculture) at the Forest Research Institute, Rotorua, New Zealand, for the investigation of fungi associated with forest trees and/or fungal taxonomy—The High Commissioner for New Zealand, 415 Strand, London, W.C.2, quoting Ref. B.13/8/1 (April 29).

ASSISTANT LECTURER OR LECTURER IN DENTAL BIOCHEMISTRY—The Registrar, The University, Liverpool, quoting Ref. CV/N (April 30).

ASSISTANT DIRECTOR (with a recognized degree or equivalent qualification, and a wide experience in agricultural botany or in crop husbandry)—The Director, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (May 1).

RESEARCH FELLOW (with a minimum of three years postgraduate research experience) IN THE DEPARTMENT OF CHEMISTRY, University College, Ibadan, Nigeria, to join a group working on the chemistry of the West African Flora, in particular to investigate the bitter principles of the West African Cucurbitaceae—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 1).

RESEARCH ASSISTANT IN DENTAL BIOCHEMISTRY, to pursue investigations into the metabolism of bone and teeth—The Registrar, The University, Liverpool, quoting Ref. CV/N (June 30).

ASSISTANT LECTURER, Grade B (with a degree or equivalent qualification, teaching experience, and able to teach to Higher National Certificate standard—The Clerk to the Governors, South-East Essex Technical College, Longbridge Road, Dagenham, Essex.

ASSISTANT LECTURER (preferably with a Ph.D. or equivalent postgraduate research experience, and a knowledge of electronics or radiochemistry) IN PHYSICAL CHEMISTRY—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

ASSISTANT PROFESSOR (PSYCHOLOGIST) IN THE UNIVERSITY DEPARTMENT OF PSYCHIATRY—The Dean of Medicine, University of Alberta, Edmonton, Alberta, Canada.

BIOLOGY TECHNICIAN, to work at the Royal Veterinary College assisting a research fellow of the Zoological Society of London in physiological and animal breeding work—The Assistant Secretary, Royal Veterinary College, Royal College Street, London, N.W.1.

DEMONSTRATORS (2) IN THE DEPARTMENT OF HORTICULTURE, to assist with practical classes on vegetable crops and ornamental plants respectively—Prof. O. V. S. Heath, F.R.S., University of Reading Horticultural Research Laboratories, Shinfield Grange, Shinfield, Berks.

EXPERIMENTAL OFFICER, for work on biochemical problems associated with the diseases of farm animals and the fundamental study of protein and nucleic acid biosynthesis—The Secretary, Agricultural Research Council, Field Station, Compton, near Newbury, Berks.

GRADUATE CHEMIST OR PHYSICAL CHEMIST (age 23 to 30 years), for work on techniques employed in the fractionation of proteins in human plasma. The work includes the development of laboratory procedures for use on a small production scale—The Secretary, Lister Institute of Preventive Medicine, Elstree, Herts.

LECTURER (with a good honours degree and preferably a higher degree) IN MATHEMATICS—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

LECTURER (with a special knowledge and/or experience in spectrophotometry and colorimetry) IN PHYSICAL CHEMISTRY—The Registrar (Department F), Bradford Institute of Technology, Bradford 7.

LECTURERS IN GEOGRAPHY, PHARMACY, BIOCHEMISTRY, INORGANIC CHEMISTRY, BOTANY, MATHEMATICS, PHYSICS, STATISTICS, and ZOOLOGY (COMPARATIVE ANATOMY)—The President, University College, P.O.B. 399, Addis Ababa, Ethiopia.

MASTER TO TEACH CHEMISTRY to "A" and "S" level G.C.E.—The Headmaster, The Perse School, Cambridge.

MASTER TO TEACH MATHEMATICS, very largely in the Sixth Form—The Headmaster, Bolton School, Bolton, Lancs.

MINERALOGIST (preferably with Ph.D. degree)—Department of Geology, University of New Brunswick, Fredericton, New Brunswick, Canada.

RESEARCH ASSISTANT (Senior Technician grade, with laboratory experience in chemistry or biochemistry, industrial or hospital), for interesting research in amino-acid metabolism in health and disease—Dr. Woolf, Department of the Regius Professor of Medicine, Radcliffe Infirmary, Oxford.

RESEARCH ASSISTANT (with an honours degree in physiology, physics or a biological subject), for experimental research in human lung function and to assist in teaching—The Finance Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1.

RESEARCH ENGINEER IN THE DEPARTMENT OF MATERIALS, to assist in setting up and developing a high-temperature research project—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

RESEARCH FELLOW (with a first degree in physics or metallurgy) IN THE DEPARTMENT OF MATERIALS, to undertake research in the fundamental deformation and annealing mechanisms in graphite—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

SENIOR BIOCHEMIST (with an appropriate science degree or the A.R.I.C.) within the Nottingham No. 2 Group of Hospitals, to work under the direction of the Chemical Pathologist at the City Hospital—The Group Secretary, Nottingham No. 2 Hospital Management Committee, Sherwood Hospital, Nottingham.

SENIOR TECHNICIAN or TECHNICIAN (with instrument or physics workshop experience) IN THE RADIOTHERAPY DEPARTMENT, Oldchurch Hospital, for duties which include Physics Workshop, Isotope Laboratory, Radium and Mould Room work—The Group Secretary, Romford H.M.C., Oldchurch Road, Romford, Essex.

SENIOR TECHNICIAN (with experience of normal histological techniques), for work with an electron microscope—The Secretary, Institute of Ophthalmology (University of London), Judd Street, London, W.C.1.

TECHNICAL ASSISTANT (female) IN THE PHYSICS DEPARTMENT, for duties concerned with the medical applications of radioactive isotopes (training will be given in the specialized techniques involved)—The Secretary, Leicester Royal Infirmary, Leicester.

TECHNICIAN (preferably with previous experience in histological techniques) IN THE RESEARCH DEPARTMENT—The Secretary, Institute of Ophthalmology (University of London), Judd Street, London, W.C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Association of British Chemical Manufacturers. A Report of Meetings held in London and Manchester on 10th and 15th June, 1960, concerning Origin Rules, Certification, and Segregation of Stocks, by the Association of British Chemical Manufacturers. Pp. iii+42. (London: Association of British Chemical Manufacturers, 1960.) 3*s.* 6*d.* [111

Report of the Seventh Commonwealth Entomological Conference, 6th–15th July, 1960. Pp. iii+399. (London: Commonwealth Institute of Entomology, 1960.) 50*s.* [111

National Union of Teachers. University Entrance: The Basic Facts. Pp. 64. (London: National Union of Teachers, 1961.) 4*s.* [111

Royal Institute of Chemistry. Lectures, Monographs and Reports, 1960. No. 3: Energy Exchange in Interactions Between Ions and Molecules. By V. N. Kondratiev. Pp. ii+16. 4*s.* net. No. 4: Chemistry in Everyday Life—The Contribution of the D.S.I.R. By Sir Harry Melville. Pp. 16. 4*s.* net. No. 5: Sugar and Its Industrial Applications. By Dr. L. F. Wiggins. Pp. ii+44. 5*s.* 6*d.* net. (London: Royal Institute of Chemistry, 1960.) [111

British Association for Commercial and Industrial Education. Policy in Perspective: Report of the BACIE Annual Conference held at Cambridge, 22–24 September, 1960. Pp. 126. (London: British Association for Commercial and Industrial Education, 1960.) 10*s.* [111

The Incandescent Heat Company, Ltd. Bulletin No. V. 10C: A Complete Service in Industrial Heat Engineering. Pp. 29. (Smethwick, Birmingham: The Incandescent Heat Company, Ltd., 1961.) [111

Kodak Limited. About Kodak. Pp. 24. (London: Kodak Limited, 1961.) [111

Association of Universities of the British Commonwealth. Report of the Executive Council, together with the Accounts of the Association for the year 1st August, 1959, to 31st July, 1960. Pp. 30. (London: Association of Universities of the British Commonwealth, 1960.) [111

Coal Tar Research Association. Review of Coal Tar Technology, January–June, 1960. (Vol. 12, Part 1.) Pp. ii+63. (Gomersal, Leeds: Coal Tar Research Association, 1960.) [191

Ministry of Power. Dust Prevention and Suppression. Instructional Pamphlet No. 3: The Application of Water to Machine Undercutting and to Deep Hole Extra High Pressure Infusion of Coal Seams. Pp. iii+8+2 plates. (London: H.M. Stationery Office, 1960.) 1*s.* 6*d.* net. [191

Commonwealth Mycological Institute. Mycological Papers, No. 79: Dematiaceous Hyphomycetes, II. By M. B. Ellis. Pp. 23. (Kew: Commonwealth Mycological Institute, 1961.) 7*s.* net. [191

Department of Scientific and Industrial Research. D.S.I.R. Studentships and Fellowships 1961. Pp. 28. 1*s.* 9*d.* net. D.S.I.R. Research Grants, 1961. Pp. iv+8. 1*s.* net. (London: H.M. Stationery Office, 1961.) [191

Department of Scientific and Industrial Research. Memoirs of the Geological Survey, Scotland. The Geology of Ben Nevis and Glen Coe and the Surrounding Country. By E. B. Bailey and H. B. Maufe. Second (revised) edition by Sir E. B. Bailey, with Economic Chapter by T. R. M. Lawrie. Pp. x+307+13 plates. (London: H.M. Stationery Office, 1960.) 35*s.* net. [191

Society for Analytical Chemistry. Report of the Analytical Methods Committee, 1959. Pp. vi+27. (London: Society for Analytical Chemistry, 1960.) [191

Other Countries

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United States Department of the Interior: Geological Survey. Water-Supply Paper 1302: Compilation of Records of Surface Waters of the United States through September 1950. Part 1-B: North Atlantic Slope Basins, New York to York River. Prepared under the direction of J. V. B. Wells. Pp. v+679+plate 1. Water-Supply Paper 1304: Compilation of Records of Surface Waters of the United States through September 1950. Part 2-B: South Atlantic Slope and Eastern Gulf of Mexico Basins, Ogeechee River to Pearl River. Prepared under the direction of J. V. B. Wells. Pp. v+399+plate 1. Water Supply Paper 1315-B: Compilation of Records of Surface Waters of the United States through September 1950. Part 11-A: Pacific Slope Basins in California except Central Valley. Prepared under the direction of J. V. B. Wells. Pp. v+461–874+plate 2. Water-Supply Paper 1420: Floods of August-October, 1955, New England to North Carolina. By Dean B. Bogart. Pp. ix+854. (Washington, D.C.: Government Printing Office, 1960.) [111

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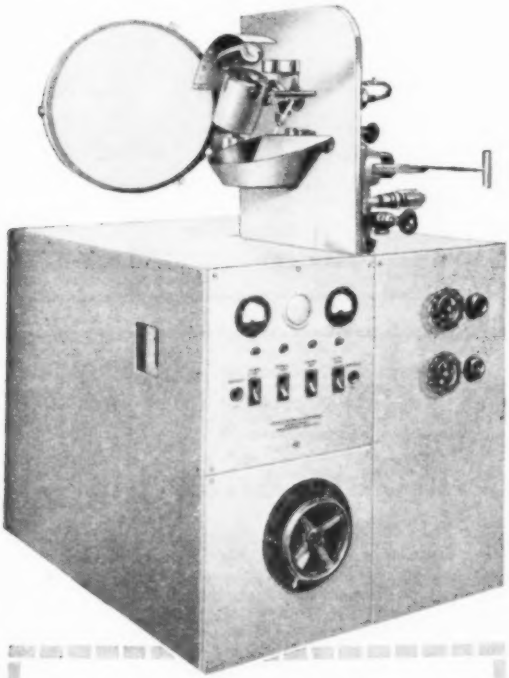

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UNIVERSITY OF EDINBURGH

DEPARTMENT OF PHYSIOLOGY

Applications are invited for the post af Lecturer in the Department of Physiology. Salary scale: £1,050 by £100 to £1,450; Bar: £1,600 by £100 to £1,900 per annum, with placement according to qualifications and experience, and with superannuation benefit and family allowance where applicable. Some experience of research on the cardiovascular system in man or animals an advantage. Candidates should have an honours degree in physiology or an allied subject, or equivalent training. The successful candidate will be expected to take up duty on October 1, 1961.

Further particulars may be obtained from the undersigned, with whom applications (six copies), giving the names of two referees should be lodged not later than May 1, 1961.

CHARLES H. STEWART,
Secretary to the University.

D.S.I.R., ROAD RESEARCH LABORATORY, Harmondsworth, Middlesex, requires Senior Scientific/Scientific Officers and Experimental/Assistant Experimental Officers (Civil Engineers, Physicists and Mathematicians) for work on the following: (1) Assessment, measurement and analysis of strains, stresses and deformations imposed on bridge and flyover structures of various types and on elevated roads by traffic loading, impact, settlement and climatic variation. (2) Effects of vibration and fatigues. (3) Study of design methods of complex bridge structures. (4) Design of bearings and hinges, parapets, joints, etc. (5) Maintenance problems. (6) Strength, elastic and rheological properties of bridge-making materials. Electronic and other techniques will be applied and a computer available where necessary. Qualifications. Scientific Officer Grades: first- or second-class honours degree or equivalent in appropriate subject. Senior Scientific Officer: at least three years appropriate postgraduate research experience required. Assistant Experimental Officer (age 22 or over); Experimental Officer (age 26 or over). Pass degree or equivalent in appropriate subject. Salary: Scientific Officer, £773 to £1,267; Senior Scientific Officer, £1,392 to £1,714. Normal prospects of promotion in mid-thirties to Principal Scientific Officer (£1,776 to £2,478). Assistant Experimental Officer, £680 (age 22) to £1,023; Experimental Officer, £1,132 to £1,386.—Forms from Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting E.77/1A.

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CONDITIONS: Under specified conditions first-class fares for successful applicant and dependants. Salary will be paid from date of embarkation.

APPLICATIONS, which close on May 1, 1961, should be made on forms obtainable from and returned to the undersigned:

Official Secretary (Ref. 61/2083), Australia House, Strand, London, W.C.2.

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Applications are invited from highly qualified graduates (mathematicians, chemists, physicists) to join a team exclusively devoted to exploring and establishing an adequate conceptual basis for the automation of industrial processes. The Automation Analysis team is a separate department of Elliott Brothers (London) Limited, and its function is to clarify the logical relationships between process variables in complex processes such as are found in oil and chemical industries.

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Applications should be addressed to:

The Personnel Manager (Ref. 347),
ELLIOTT BROTHERS (LONDON) LIMITED,
Elstree Way, Borehamwood, Herts.

RESEARCH FELLOWSHIP IN PHARMACOLOGY

On the retirement of Professor J. H. Burn from the Chair of Pharmacology at Oxford in 1959 his colleagues contributed to a Trust Fund to commemorate his work. Applications are now invited for the post of Burn Research Fellow in Pharmacology at Oxford. Applicants should be less than 30 years old and should be graduates with two to three years research experience. The Fellowship will be tenable for one year from October 1, 1961, and would be renewable for a further two years at the discretion of the Trustees. The remuneration will be £800 to £1,000 per annum.

Applications to the Secretary to the Trustees, Department of Pharmacology, University of Oxford, before May 15, 1961.

ONTARIO CANCER FOUNDATION

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HAMILTON GENERAL HOSPITAL,
HAMILTON, ONTARIO

Applications are invited for appointment as Clinical Physicist. Applicants should possess at least an honours degree in physics or equivalent. Some experience in hospital physics is desirable but not necessary. An interest in research in clinical physics or radiobiology is expected. Minimum initial salary for untrained honours graduate is $5,000 per annum; pension scheme.

Applications should be sent to the undersigned, from whom further details may be obtained.

R. J. HORSLEY,
Senior Physicist.

MONASH UNIVERSITY
CLAYTON, VICTORIA, AUSTRALIA
CHAIR OF PHYSIOLOGY

The Interim Council of Monash University invites applications to the Foundation Chair of Physiology. The University opened on March 13, 1961, with Faculties of Medicine, Arts, Economics and Politics, Science and Engineering, at Clayton, 14 miles from the centre of Melbourne. Students who began the first year of their medical course in March, 1961 will start physiology in March, 1962. Planning for the first portion of the Medical School to be built by the end of 1962 is proceeding, and multi-discipline laboratories for Medical Biochemistry, Physiology, Pharmacology and Histology will be incorporated. Biochemistry, other than Medical, will be taught in the Chemistry School. The successful applicant will have the opportunity to take part in the planning of his department and, if from overseas, air fares will be paid for this purpose so that, immediately on appointment, a short visit for consultation with the Dean of the Faculty and the Architects can take place. For the building of the first phase (1961-63) of the Medical School (Anatomy, Medical Biochemistry and Physiology) the Australian Universities Commission has allotted £500,000. The first three years of the course will be at the University, and the three clinical years at Alfred Hospital (with its associated Baker Medical Research Institute) and Prince Henry's Hospital. The Chairs of Pathology and Microbiology will be at Alfred Hospital and the Clinical Chairs at the two hospitals. A site next to the Medical School has been reserved at the University for a General Teaching Hospital which, it is expected, will be built in the next ten years. The successful applicant will be a full-time officer of the University and will be expected (i) to teach, conduct examinations and exercise supervision over the work of his Department in accordance with the University Statutes and Regulations and the direction of the Interim Council to whom he will be responsible through the Vice-Chancellor, (ii) to carry out and organize research amongst staff and postgraduate students and (iii) to advise the Interim Council on the establishment and development of his own Department. The salary of the Professor will be subject to negotiation but will be not less than £A.4,000 per annum. Superannuation provision is similar to F.S.S.U., there will be travelling and removal expenses to Melbourne for the appointee and his family.

Further information and details of the terms and conditions of appointment for the Chair may be obtained from the undersigned with whom applications close on June 10, 1961, or from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1.

F. H. JOHNSON,
Registrar.

Monash University,
Clayton, Victoria, Australia.

UNIVERSITY OF HONG KONG
ASSISTANT LECTURESHIPS IN APPLIED OR PURE MATHEMATICS

Applications are invited for the posts of Assistant Lecturers in Applied or Pure Mathematics. Applicants should have appropriate qualifications and teaching and research experience. Annual salary (superannuable) is £1,375 by £50 to £1,525 for a man or £1,040 by £40 to £1,160 for a woman. There is also an expatriation allowance of £225 a year if applicable; salaries are under review. The equivalent of income tax in the Colony is comparatively low. First-class sea passages are provided for expatriate staff and their families on first appointment and leaves. Accommodation at reasonable rental is provided for those receiving expatriation allowance.

Further particulars and information as to the method of application should be obtained from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close, in Hong Kong and London, on May 19, 1961.

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Applications, quoting Ref. 17/39, giving full details, including age and experience, together with names of two referees and/or copy of testimonials to the Group Secretary, Romford H.M.C., Oldchurch Road, Romford.

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Applications should be forwarded to: Chief Chemist, Gallaher Limited, Virginia House, 138 York Street, Belfast, 15.

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Applications to Hospital Secretary, King's Mill Hospital, Sutton-in-Ashfield, Notts.

JOHN INNES INSTITUTE

Applications are invited from biochemists, biophysicists and physical chemists with an interest in biological problems for positions on the staff of the new Cell Biology Department at the John Innes Institute. The work of the laboratory is broadly based on problems in the physiology and biochemistry of animal and plant cells. Current investigations include studies on the biology and chemistry of nucleic acids, on nucleo-cytoplasmic interrelationships and on factors controlling cell multiplications. The appointments are at various levels and will interest both established investigators and those who might profit by a period of collaboration with senior workers.

Inquiries should be addressed to the Head of the Department of Cell Biology, John Innes Institute, Bayfordbury, Hertford, Herts, from whom further information may be obtained.

COLLEGE OF AERONAUTICS

Applications are invited for a Research Fellow in the Department of Materials to undertake research on the fundamental deformation and annealing mechanisms in Graphite. Candidates should possess a first degree in physics or metallurgy, and the person appointed will be permitted to submit his work for a higher degree. Salary up to £1,500 per annum, depending on qualifications and experience, with superannuation under F.S.S.U. and family allowance.

Appointment will be made initially for three years with prospects of continuation. Applications, containing full particulars should be addressed to the Recorder, the College of Aeronautics, Cranfield, Bletchley, Bucks.

RESEARCH FELLOWSHIPS

R.A.E. Ministry of Aviation invites applications for a limited number of Senior and Junior Research Fellowships tenable for three years at the Royal Aircraft Establishment, Farnborough, Hants, or Bedford.

Fellows will be required to carry out Fundamental Research in connection with advanced rocket and aircraft projects. The scientific fields covered include aerodynamics, thermodynamics, aircraft and missile structures, guidance and control techniques, new synthetic polymers and their properties, electronics involving solid state physics, semi-conductors, propagation, physics and chemistry of surfaces, gas discharge physics, microwave physics, etc. Subjects will be selected according to the training and experience of the Fellows and the needs of the Establishment. Candidates must be British subjects with normally either a first- or second-class honours degree in the natural sciences, mathematics or engineering, and with evidence of a very high standard of ability in research. Exceptionally a candidate without the academic requirements but who can produce evidence of outstanding ability in research may be admitted. At least two years' postgraduate research experience required for Junior Research Fellowships (age preferably 23 to 26) and at least three years' for Senior Research Fellowships (age preferably 26 to 30).

Remuneration according to individual merit on ranges £1,325 to £1,640 (Senior Research Fellowships), £910 to £1,220 (Junior Research Fellowships). Superannuation under F.S.S.U. At expiry of a Fellowship the holder, if of suitable age, can be considered for an established (pensionable) post through a Civil Service Commission Open Competition.

Application forms and booklet obtainable from the Director, R.A.E., Farnborough, Hants, for return by April 24, 1961. Overseas candidates should send written applications, stating age, nationality and place of birth of self and parents, educational and academic qualifications, appointments held and research experience.

CRYSTAL GROWTH

National Physical Laboratory, Teddington, Middlesex, requires Senior Scientific Officer/Scientific Officer to investigate techniques of high temperature recrystallization, zone melting, and hydrothermal synthesis. High purity single crystals of synthetic rubs and associated materials are required for LASER applications. Qualifications: first- or second-class honours in physics, chemistry, or metallurgy, or equivalent. An interest in physical chemistry, solid state physics or crystallography is desirable. At least three years postgraduate research experience required for Senior Scientific Officer. Salary: Scientific Officer, £773 to £1,267; Senior Scientific Officer, £1,392 to £1,714. Also an Assistant Experimental Officer to assist in this field. Qualifications: Pass degree, H.N.C. in physics, chemistry, or metallurgy or equivalent. At least age 26 for Experimental Officer. Salary: Assistant Experimental Officer, £680 (age 22), £841 (age 26) to £1,023; Experimental Officer, £1,132 to £1,386.

Forms from Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting F155/1A (Assistant Experimental Officer/Experimental Officer post) or F156/1A (for Scientific Officer/Senior Scientific Officer). Closing date April 24, 1961.

AGRICULTURAL RESEARCH COUNCIL require, at their Radiobiological Laboratory: (a) Scientific Officer, for three years in the first instance, to carry out and direct measurements of artificial and natural radio elements in plants, soils and foods; and to develop new techniques. Good second-class honours degree in chemistry required. Salary in scale £717 to £1,222, F.S.S.U. (b) Two Assistant Experimental Officers or Experimental Officers; one for radio chemistry and one for the design, operation and maintenance of low level counting equipment. (c) Assistant (Scientific) or Assistant Experimental Officer for statistical analyses and other computations of experimental results. Minimum qualifications: Assistant Experimental Officer under 22, G.C.E. in five subjects, including two mathematical or scientific, at advanced level; over 22, pass degree or equivalent. Starting salary £450 (age 18) to £776 (over 26) rising to £957. Experimental Officer, for experienced candidates over 28, £1,057 rising in scale to £1,336. Assistant (Scientific), G.C.E. in four subjects, including English and Mathematics, at ordinary level, or equivalent. Starting salary in scale £325 (age 16) to £531 (25 or over) rising to £723. Contributory superannuation scheme.—Applications, with names of two referees, to Secretary of the Laboratory, Letcombe Regis, Wantage, Berks, within two weeks.

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Aldermaston Court,
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UNIVERSITY OF SYDNEY

READERSHIP AND WILLIAM McILRATH RESEARCH FELLOWSHIP IN HUSBANDRY

Applications are invited for the position of Reader in Animal Husbandry which has recently been established within the University of Sydney under a grant from the Commonwealth Scientific and Industrial Research Organization. The successful applicant will also be appointed as William McIlrath Research Fellow. Research experience in a field dealing with the production, processing and marketing of meat and meat products is essential. The appointee will be responsible, under the general direction of the Professor of Animal Husbandry, for the administration of the Meats Research Laboratory at Camden. He will be expected to conduct courses in Meat Production and Technology and to direct a major part of the research programme in the meats field and in particular to undertake research in the problems of beef production from dairy herds. The salary will be £A.3,300 per annum, plus cost of living adjustments (at present £A.36 per annum) and will be subject to deductions under the State Superannuation Act. Under the Staff Members' Housing Scheme, in cases approved by the University and its Bankers, married men may be assisted by loans to purchase a house.

Further particulars and information as to the method of application may be obtained from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close, in Australia and London, on May 19, 1961.

ST. MARY'S HOSPITAL

PADDINGTON, W.2

Senior Technician or Technician required in the Clinical Isotope Laboratory. Whitley Council salary and conditions of service.

Applications, giving full details of experience in isotope work and/or electronics, together with the names of two referees, to Alan Powditch, House Governor.

UNIVERSITY OF ADELAIDE

TECHNICAL ASSISTANT OR LABORATORY ASSISTANT

Applications are invited for the above-mentioned appointment in the Department of Botany. Salary scales: Technical Assistant, male, £A.1,095 by £A.35 to £A.1,305, female, £A.823 by £A.26 to £A.979; Laboratory Assistant, male, £A.885 by £A.35 to £A.1,095, female, £A.665 by £A.26 to £A.821. Qualifications: General laboratory experience and competence with apparatus and techniques in biology, and preferably with preparation and collection of botanical teaching materials. Duties: Supervision of basic laboratory apparatus and preparation of undergraduate classes. General: (a) No provision is made for travelling expenses, but arrangements could be made to help a candidate from the United Kingdom to secure an assisted passage under the Commonwealth Migration Scheme. (b) Preference will be given to a qualified male applicant, but women also are invited to apply. (c) Any further information desired will be supplied on request to the Registrar.

Applications, giving particulars of qualifications and experience, marital status, the names and addresses of two referees, and the time when the applicant would be free to assume duty, should be sent to the Registrar, The University of Adelaide, Adelaide, South Australia, not later than May 15, 1961.

MONASH UNIVERSITY

CLAYTON, VICTORIA, AUSTRALIA
SENIOR LECTURER OR LECTURER IN CHEMISTRY

Applications are invited for appointment to the position of Senior Lecturer or Lecturer in Chemistry. Commencing salary will be determined in accordance with qualifications and experience within the ranges of £A.2,480 to £A.2,950 (Senior Lecturer) or £A.1,660 to £2,360 (Lecturer). The main research interests of the Department are theoretical chemistry, microwave, infrared and ultraviolet spectroscopy, NMR spectroscopy, physical-organic chemistry, structural studies relative to antibiotics, development of new organic reactions, inorganic reaction mechanisms, studies of volatile inorganic compounds. Preference may be given to candidates with research interests in the field of NMR, physical-organic chemistry, or inorganic chemistry.

Further information may be obtained from the Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia, to whom inquiries of a general nature should be addressed, or from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Technical inquiries should be addressed to the Head of the Chemistry Department, Professor R. D. Brown. Applications close on May 15, 1961.

ROYAL COLLEGE OF SCIENCE AND TECHNOLOGY

GLASGOW
DEPARTMENT OF ELECTRICAL ENGINEERING

Applications are invited for two Lectureships on salary scale £1,050 to £1,850, with F.S.S.U. and Family Allowances. Initial salary will have regard to qualifications and experience. 1. Lecturer in Telecommunications—candidates must have had experience in this field. 2. Lecturer in Electrical Technology—candidates should have some experience in electrical machines work. The Department offers a wide range of undergraduate courses, and has excellent facilities for research.

Forms of application may be obtained from the Secretary, The Royal College of Science and Technology, Glasgow, C.1.

UNIVERSITY OF NATAL

DEPARTMENT OF CHEMISTRY

Applications are invited from suitably qualified persons for appointment to the posts of: Senior Lecturer in Inorganic Chemistry, Durban. Lecturer in Inorganic Chemistry, Pietermaritzburg. The salary scales attached to the posts are: Senior Lecturer, R.3,180 by R.120 to R.4,140 (£1,590 by £60 to £2,070); Lecturer, R.2,340 by R.120 to R.3,300 (£1,170 by £60 to £1,650). The commencing notch on these scales will be dependent on the qualifications and/or experience of the successful candidates. The University reserves the right to appoint someone other than from those who make application.

Application forms and further particulars are obtainable from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close, in South Africa and London, on April 30, 1961.

NORTHAMPTON COUNTY BOROUGH EDUCATION COMMITTEE

NORTHAMPTON COLLEGE OF TECHNOLOGY
ST. GEORGE'S AVENUE, NORTHAMPTON

Applications are invited for the following full-time teaching appointments, duties to commence September 1, 1961:

1. Grade B Assistant in Chemistry. Applicants should be graduates or hold equivalent qualifications and be able to teach one branch of Chemistry to General Degree standard. An ability to teach Physics up to advanced level will be considered an advantage.

2. Grade A Assistant in Physics and Chemistry or Biology. Applicants should be graduates or hold equivalent qualifications and be able to teach Physics up to advanced level standard. An ability to teach elementary Chemistry or Biology would be considered an advantage.

Salaries in accordance with the Burnham Technical Report, with allowances for training, industrial and teaching experience. Scales: Grade B, £700 by £27 10s. to £1,150; Grade A, £520 by £27 10s. to £1,000.

Forms of application and further particulars may be obtained from the undersigned, to whom applications should be returned within two weeks of the appearance of this advertisement.

H. A. SKERRETT,
Chief Education Officer.

Education Office, Springfield,
Cliftonville, Northampton.

LEEDS REGIONAL HOSPITAL BOARD

REGIONAL PHYSICS UNIT

Applications are invited for the appointment of a Hospital Physicist for duties with the Regional Physics Unit whose Headquarters are at Cookridge Hospital, Leeds. The post will be either in the basic or senior grade according to experience. The work is mainly concerned with the treatment of disease by High Energy Units, but opportunities also exist for work with radio-isotopes and co-operation with medical staff on other physical problems. Candidates should possess an appropriate honours degree, and for appointment to the Basic Grade need have had no previous experience, but for the Senior Grade not less than five years' experience as a Hospital Physicist is required. New entrants in the Basic Grade will be required to undergo a probationary period of at least two years. Salary scales: Basic Grade Probationary, £730 to £925 per annum. Post-Probationary, £925 to £1,150 per annum. Senior Grades, £1,250 to £1,700 per annum.

Applications, stating age, qualifications, experience, and details of present and previous appointments, together with dates and the names and addresses of two referees, to the Secretary, Park Parade, Harrogate, by not later than April 17, 1961.

DERBY AND DISTRICT COLLEGE OF TECHNOLOGY

Principal: T. Heap, Ph.D., M.Sc., F.R.I.C., A.M.B.I.M.

Applications are invited for the post of Assistant Grade B in Biology. Applicants should possess a university degree and preferably have some teaching experience, especially of G.C.E. "O" level courses in Biology. Ability to teach elementary Human Anatomy, Physiology and Hygiene would be an advantage. Salary in accordance with the Burnham Further Education Scale: £700 by £27 10s. to £1,150, with additions for training and graduate qualifications. Up to 12 increments on the scale may be allowed for approved industrial experience.

Application form, returnable by April 21, and further particulars may be obtained from the Registrar, Derby and District College of Technology, Kedleston Road, Derby.

UNIVERSITY OF READING

DEPARTMENT OF CHEMISTRY

Applications are invited for one or more Research Assistantships for experimental work on the thermodynamic properties of gases and of liquid mixtures. Candidates should have a degree in chemistry or chemical engineering or should have equivalent qualifications such as Grad.R.I.C. The commencing salary will be not less than £475 per annum depending on age and experience. Research Assistant will be able to apply to register for a higher degree.

Applications should be sent as soon as possible to the Bursar, the University, Reading.

UNICAM INSTRUMENTS LIMITED

PHYSICISTS

The research and development department of UNICAM INSTRUMENTS LTD. has a number of vacancies for graduate physicists with an interest in spectroscopic instruments in the ultraviolet, infrared and radio-frequency fields. A good honours degree is required, postgraduate research or engineering experience would be an advantage, and the ability to lead a small project team will be necessary. The salary offered will be appropriate to qualifications and experience.

Please reply to Unicam Instruments Ltd., Arbury Road, Cambridge, quoting reference E.D.34.

UNITED KINGDOM ATOMIC ENERGY AUTHORITY

PHYSICISTS & CHEMISTS

required as Reactor Operating Staff at Dounreay Experimental Reactor Establishment, Thurso, Caithness, Scotland.

Successful applicants will be required to take control of a shift team responsible for the safe and efficient operation of the high flux Materials Testing Reactor, its ancillary plant and the experimental test rigs under irradiation in it. More junior posts are also available for younger men whose duties will cover a wide field of interests in reactor operations.

Salaries assessed according to age, qualifications and experience from £1,005 to £2,090 per annum.

Applicants should hold an honours degree or equivalent in physics or chemistry with at least three years' relevant experience, or be Associates of the Royal Institute of Chemistry.

Rented housing and house purchase assistance available for married officers living beyond daily travelling distance.

Hostel accommodation available.

Contributory superannuation.

Send postcard for application form to the Personnel Manager, D.E.R.E., Dounreay, Thurso, Caithness, Scotland, quoting reference 214/34.

BASUTOLAND

PIUS XII COLLEGE, ROMA, BASUTOLAND
LECTURESHIP OR ASSISTANT LECTURESHIP IN ZOOLOGY

Applications are invited for a Lectureship or Assistant Lectureship in Zoology. Candidates interested in Genetics and Ecology would be preferred. Basutoland offers excellent opportunities for research in these fields. Appointment tenable from February 1, 1962. Salary scale for Lecturer, £1,000 by £50 to £1,250 (efficiency bar) by £50 to £1,550; for Assistant Lecturer, £720 by £40 to £960. Status and initial salary according to qualifications and experience. Pension scheme. Family allowance of £100 per annum, plus £40 per child under the age of 18. Train and sea passages arranged for appointee, wife and children. Baggage allowance.

Further information and application forms, to be returned by May 31, obtainable from the Registrar, Pius XII College, Roma, Basutoland, Southern Africa.

UNIVERSITY OF HULL

Applications are invited for appointment to an Assistant Lectureship in Physical Chemistry tenable from October 1, 1961. Salary £800 to £950 per annum, with F.S.S.U. and family allowances.

Further particulars may be obtained from the undersigned, to whom applications (six copies) should be sent not later than April 26, 1961.

W. D. CRAIG,
Registrar.

SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH

PNEUMOCONIOSIS RESEARCH UNIT, JOHANNESBURG, SOUTH AFRICA

Vacancies exist for Senior Technicians in the Departments of Histopathology, Bacteriology and Serology. Applicants should be Associates of the Institute of Medical Laboratory Technology or Registered as Medical Technologists with the South African Medical and Dental Council. The work involved is primarily of a research nature, but the successful candidates must be prepared to assist in the routine work of the Unit if required. The commencing salary will be determined according to qualifications and experience in the scale £700 by £50 to £900 by £60 to £1,020; £1,020 by £60 to £1,200; £1,200 by £60 to £1,500 for males and £540 by £30 to £720 by £40 to £840; £800 by £40 to £1,000; £960 by £40 to £1,080 by £60 to £1,320 for females. Membership of the Staff Provident Fund and Medical Aid Scheme is compulsory and for this purpose a medical certificate of sound health is required. The travelling expenses of successful overseas applicants will be met on a pre-arranged basis.

Applications, giving full details of training, qualifications and experience, should be submitted to the Director, Pneumoconiosis Research Unit, Box 1038, Johannesburg, from whom further details may be obtained.

WELSH COLLEGE OF ADVANCED TECHNOLOGY

DEPARTMENT OF APPLIED PHYSICS

Applications are invited for the post of

SENIOR LECTURER IN PHYSICS

Applicants should have a good honours degree in physics, good teaching experience, industrial or research experience, and should be able to teach physics to final year Dip.Tech. standard. Applications from candidates who are specialists in some branch of Applied Physics would be particularly welcome. Salary scale: £1,550 by £50 to £1,750 per annum. Every encouragement and assistance will be given to the successful candidate to pursue a research project; research in the Department is largely concerned with various aspects of Solid State Physics.

Further particulars and application forms can be obtained from the Registrar at the College, Cathays Park, Cardiff, to whom they should be returned by April 26, 1961.

ROBERT E. PRESSWOOD,
Clerk to the Governors.

City Hall, Cardiff.

SOUTH-WEST ESSEX TECHNICAL COLLEGE AND SCHOOL OF ART

FOREST ROAD, WALTHAMSTOW, E.17

Senior Lecturer in Applied Biochemistry required as soon as possible. The person appointed will be responsible for the teaching of Biochemistry and related subjects, paper chromatography, low and high voltage paper electrophoresis, and vapour phase chromatography, to H.N.C., B.Sc., and Grad.R.I.C. students and in the Laboratory Technician courses as well as in the special and post-graduate courses. Experience in the field of nucleic acid chemistry, enzyme chemistry, or radioactive tracer techniques, or interest in Electronic Instrumentation, is an added recommendation. Candidates should possess high academic qualifications, have teaching experience and provide evidence of research ability. Considerable research facilities are available and fundamental research will be encouraged. Salary within scale £1,550 to £1,750 per annum, plus £38 or £51 London allowance.

Apply by letter only (no forms) to the Clerk to the Governors, giving details of qualifications, training and experience, together with three recent testimonials or the names and addresses of three referees.

UNIVERSITY OF LEEDS

DEPARTMENT OF ZOOLOGY

Applications are invited from honours graduates in zoology or physiology for appointment as Lecturer or Assistant Lecturer in the Department of Zoology at a salary on the scale £1,050 by £50 to £1,400 by £75 to £1,850 (efficiency bar at £1,550) a year for a Lecturer, or within the range £800 to £950 a year for an Assistant Lecturer, according to age, qualifications and experience. Research facilities available include those of the University's Marine Biological Station at Robin Hood's Bay.

Applications (three copies), stating date of birth, qualifications and experience, together with the names of three referees, should reach the Registrar, The University, Leeds, 2 (from whom further particulars may be obtained), not later than May 1, 1961.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

RESEARCH PHYSICIST

The Organization's Division of Metrology, National Standards Laboratory, Sydney, N.S.W., invites applications for the position of Research Physicist in the Interferometry Section from Honours graduates, majoring in Physics (or equivalent qualifications) with some years of postgraduate research experience.

The appointee is required for research in interference spectroscopy and the development of new forms of light sources. These sources may include atomic beams and other types of sources that emit intense and highly monochromatic radiation suitable for interferometry at large path differences. The Section has a well-equipped vacuum coating laboratory and is also engaged in making isotopic light sources.

Salary: Dependent on qualifications and experience within the ranges £A 1,510 to £A 2,265 per annum, or £A 2,395 to £A 2,720 per annum.

Salary for a woman will be £A 180 per annum less than corresponding rates for men.

Promotion within C.S.I.R.O. is by merit and may ultimately go beyond the upper limit of the scale within which the original appointment is made.

Fares paid. Further particulars of conditions, etc., supplied on application to:

Mr. E. J. Drake, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No. 750/222) should be addressed by May 13, 1961.

UNIVERSITY OF ADELAIDE

DEMONSTRATOR IN BOTANY

Applications are invited for the above-mentioned appointment. Salary scale: £A 1,075 by £A 45 to £A 1,300. General: (a) The appointment will be until the end of 1962 in the first instance and then subject to annual renewal. No provision is made for superannuation or travelling expenses, but arrangements could be made to help a candidate from the United Kingdom to secure an assisted passage under the Commonwealth Migration Scheme. (b) There are ample facilities for proceeding to higher degrees as about half-time during term and practically full-time during vacations is available for a Demonstrator's own advanced studies and research. (c) Any further information desired will be supplied on request to the Registrar.

Applications, giving particulars of academic record and experience (if any), the names and addresses of two referees, and the time when the applicant would be free to assume duty, should be sent to the Registrar, The University of Adelaide, Adelaide, South Australia, not later than May 15, 1961.

UNIVERSITY COLLEGE OF SOUTH WALES AND MONMOUTHSHIRE

Applications are invited for Research Demonstrator in the Department of Geology. Experience or interest in stratigraphy, structure and/or stratigraphical palaeontology required. The appointment will be on a yearly basis, renewable up to a maximum of three years. Salary £750 per annum.

Applications (two copies), giving the names of three referees, should be sent to the Registrar, University College, Cardiff, by May 5, 1961.

MEDICAL RESEARCH COUNCIL OF IRELAND

Applications are invited from B.Sc. graduates or Associates of Institute of Medical Laboratory Technology, for a position in a clinical metabolic unit (St. Vincent's Hospital, Dublin). Applicants should preferably, but not necessarily, have previous experience of this type of work, and some knowledge of radioisotope techniques. Salary offered depends on qualifications and experience.

Applications, giving full details and names of two referees, should reach the Secretary, Medical Research Council of Ireland, 50 Merrion Square, Dublin 2, on or before April 17, 1961.

UNIVERSITY OF READING

DEPARTMENT OF CHEMISTRY

Applications are invited for a Research Chemist to continue a study of the thermodynamics of imperfect gases under the terms of a contract between the University and the D.S.I.R. Candidates should have experience of research work in physical chemistry or chemical engineering and should preferably have taken a higher degree in one of these subjects. The appointment will be for one year in the first instance (from a date to be arranged) but is likely to be renewed for two further years. The commencing salary will be not less than £800 per annum depending on the qualifications and experience of the successful applicant.

Applications, including names of not more than three referees, should be sent as soon as possible to the Registrar (Room 22, O.R.B.), the University, Reading.

UNIVERSITY OF LIVERPOOL

Applications are invited for the following posts in the Department of Botany:

Assistant Lecturer or Lecturer: The initial salary will be within the range £800 to £1,250 per annum, according to age, qualifications and experience. Preference will be given to a candidate in the field of taxonomy.

Demonstrator: The initial salary will be within the range £675 to £725 per annum, according to age, qualifications and experience.

Applications, stating age, academic qualifications and experience, together with the names of three referees, should be received not later than April 28, 1961, by the Registrar, from whom further particulars of the conditions of appointment may be obtained. Quoting reference CV/N.

YORK "A" AND TADCASTER HOSPITAL MANAGEMENT COMMITTEE

Senior II or Chief Technician I required to work in the Biochemistry Section of the Group Laboratory. I.M.L.T. with biochemistry essential. Applicants should also have had considerable experience in biochemistry and preferably in the more advanced techniques. Salary scale £760 by £25 (2) by £30 (5) to £960 per annum for Senior II post or £905 by £30 (6) to £1,085 per annum for Chief Technician I.

Applications, giving details of age, experience, qualifications and names of two referees, to Group Secretary, Bootham Park, York.

UNIVERSITY OF EDINBURGH

DEPARTMENT OF ELECTRICAL ENGINEERING

Applications are invited for the posts of Assistant and Lecturer in the new department of Electrical Engineering, to take up duties as soon as possible. Applicants should have a good honours degree in electrical engineering, with special interests and experience in one of the following branches: electrical machines, microwaves, plasmas, computer design techniques, solid-state electronics. New laboratories are being equipped and courses being planned which will give considerable individual responsibility to the Lecturers. Assistants would be expected to undertake research for a higher degree. Salary scales: Assistant, £800 by £50 to £950 per annum; Lecturer, £1,050 by £50 to £1,250; Bar: £1,300 by £50 to £1,400 by £75 to £1,850 per annum. Placement on the scales will be according to qualifications and experience. The posts are subject to superannuation, and family allowance is payable where applicable.

Further particulars may be obtained from the undersigned, with whom applications (six copies), giving the names of two referees, should be lodged not later than April 29, 1961.

CHARLES H. STEWART,
Secretary to the University.

UNIVERSITY OF DURHAM

KING'S COLLEGE, NEWCASTLE UPON TYNE, 1

The Council of King's College invite applications from (i) biochemists, biophysicists or physiologists with interest in higher or lower plants or micro-organisms, for the post of Lecturer and (ii) graduates in botany for the post of Demonstrator. Salary scales: Lecturer £1,050 to £1,850 per annum, plus F.S.S.U. and child allowance; Demonstrator, £750 to £850, exceptionally to £1,150, plus F.S.S.U. and child allowance. The appointment of Demonstrator will be for one year in the first instance, renewable for a further period. The appointments will be made at points on the respective scales appropriate to the qualifications and experience of the successful applicants. They will be tenable in the Department of Botany.

Applications, including names and addresses of three referees, should be sent to the Registrar and Secretary of King's College, from whom further particulars may be obtained, not later than May 6, 1961.

UNIVERSITY OF HONG KONG

LECTURESHIP IN ZOOLOGY

Applications are invited for the above-mentioned post. Applicants should possess a good honours degree in zoology, and preferably have had some experience of teaching and research. Annual salary (superannuable) is £1,575 by £50 to £2,175 for a man or £1,200 by £40 to £1,680 for a woman. There is also an expatriation allowance of £225 a year if applicable; salaries are under review. The equivalent of income tax in the Colony is comparatively low. First-class sea passages are provided for expatriate staff and their families on first appointment and leaves. Accommodation at a reasonable rental is provided for those receiving expatriation allowance.

Further particulars and information as to the method of application should be obtained from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close in Hong Kong and London, on May 30, 1961.

UNIVERSITY OF SHEFFIELD

Applications are invited for the following posts, duties to commence on October 1, 1961: An Assistant Lecturer in Statistics; a Lecturer or Assistant Lecturer in Applied Mathematics; a Lecturer in Pure Mathematics. Initial salary according to qualifications and experience on the scales: Lecturer, £1,050 by £50 to £1,400 by £75 to £1,850; Assistant Lecturer, £800 by £50 to £950, with F.S.S.U. provision and family allowance. Grants towards removal expenses will be made.

Further particulars may be obtained from the Registrar, to whom applications (four copies) should be sent by April 29, 1961.

UNIVERSITY COLLEGE LONDON (GOWER Street, W.C.1) requires Temporary Assistant Lecturer or Temporary Lecturer in Department of Mathematics from October 1, 1961. Preference will be given to applicants whose interests are in applied mathematics. Salary £800 to £1,400, plus £60 London allowance, per annum, according to qualifications and experience; family allowance.—Applications to be received by May 8, 1961, should be sent to the Secretary, from whom further particulars may be obtained.

AUSTRALIAN NATIONAL UNIVERSITY

INSTITUTE OF ADVANCED STUDIES
JOHN CURTIN SCHOOL OF MEDICAL RESEARCH
RESEARCH FELLOWSHIP IN PHYSICAL BIOCHEMISTRY

Applications are invited for a Research Fellowship in the Department of Physical Biochemistry. Research experience in biochemistry or chemistry and interest in physico-chemical aspects of biochemistry are essential. The Research Fellow will be allowed reasonable scope in the choice of his research. The salary range for Research Fellows is £A.1,437 to £A.2,152, with increments of £A.65 per annum, but this range is under review; initial salary will depend on qualifications and experience. Appointment is for three years, with possible extension to a maximum of five years. Superannuation is on the F.S.S.U. pattern. Reasonable travel expenses are paid and assistance with housing is provided. A summary of the conditions of appointment is available from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close, in Australia and London, on April 28, 1961.

Details of the research being carried out may be obtained from the Head of the Department, Professor A. G. Ogston, F.R.S.

QUEEN'S UNIVERSITY

KINGSTON, ONTARIO, CANADA
LECTURER IN CHEMISTRY

Applications are invited for the above post. Candidates should have research experience. The appointee will be expected to teach undergraduates and carry on fundamental research. The appointment is probationary for the first two years.

Applications should include names of two referees to whom application may be made for references. Details of papers published and research interests should also be given. Further particulars can be obtained from Professor J. K. N. Jones.

MIDDLESEX HOSPITAL MEDICAL SCHOOL

DEPARTMENT OF BIOLOGY AS APPLIED TO MEDICINE

Applications are invited for the post of Assistant Lecturer in the Department, the appointment to begin in October, 1961. The successful candidate will be expected to participate in the teaching of pre-medical, medical and postgraduate students, but ample opportunities will be offered for research. Preference will be given to candidates with research experience in some field of experimental zoology.

Applications, in triplicate and with the names of two referees, should be made before July 1, 1961, to the Secretary, Department of Biology as Applied to Medicine, Middlesex Hospital Medical School, London, W.1, from whom further particulars can be obtained.

STOKE MANDEVILLE HOSPITAL AYLESBURY

Assistant Biochemist required in the Area Pathology Laboratory based at the above Hospital. Applicants should possess first- or second-class honours degree in chemistry or biochemistry, or A.R.I.C. Whitley Council conditions of service. Salary £925 to £1,150 per annum after (for new entrants only) a probationary period. The post offers a wide variety of experience in a well-equipped and pleasant laboratory.

Applications, giving full details of qualifications etc., together with names of two referees, to be forwarded to the Administrative Officer as soon as possible.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

The Department of Electrical Engineering has vacancies for Lecturers in fields including: (i) electrical machines; (ii) electric traction; (iii) automatic control—mathematical aspects; (iv) electron physics—vacuum and plasma devices. The department is soon to occupy a new building with modern equipment, and research is in progress in all these fields. Candidates will, in general, be expected to participate in postgraduate teaching and research. Salaries in scale £1,050 by £50 to £1,400 by £75 to £1,550 (bar) by £75 to £1,850 per annum, plus £60 per annum London allowance, family allowances and F.S.S.U. membership. Appointments may be made in the higher part of this range in the case of suitably qualified candidates.

Apply to Head of Department of Electrical Engineering, Imperial College of Science and Technology, London, S.W.7.

U.S.A.

UNIVERSITY OF ALABAMA MEDICAL SCHOOL

GENERAL BIOCHEMIST

Biochemist required for Gastroenterology Research Laboratories with special interest in gastric secretion (electrolytes, pepsin), small bowel absorption and lipid analysis. Research experience essential. Starting salary according to experience, but would not be less than $7,000 per annum.

MEDICAL TECHNICIAN

Medical Technician required for Gastroenterology Research Laboratories with special interest in gastric secretion (electrolytes, pepsin), small bowel absorption and lipid analysis. Research experience desirable but not essential.

Applications should be addressed to:
Basil I. Hirschowitz, M.D.,
Director, Division of Gastroenterology,
University of Alabama Medical Center,
Birmingham, Alabama.

THE DISTILLERS COMPANY LIMITED

CHEMICAL ENGINEERS

The Chemical Division wish to appoint two chemical engineers in their factory and Development Department at Carshalton, Surrey.

One of the men appointed will be engaged on general design and development work in connection with the factory plant. The other man will be designing pilot plant and preparing basic design data for small works plant.

Candidates, aged not over 30, should hold a degree or its equivalent in chemical engineering, and have experience appropriate to one of the posts mentioned above. The Company operates a non-contributory pension scheme.

Write:
STAFF MANAGER
THE DISTILLERS COMPANY LIMITED
21/22 BOLTON STREET
LONDON, W.1.

Quote Ref. 20/61.N.

COLLEGE OF ADVANCED TECHNOLOGY
BIRMINGHAM

NUFFIELD RESEARCH FELLOW

Applications are invited for this post in the Department of Industrial Administration, which is for planning and executing an investigation of the spacing of college and industrial training in "sandwich" courses, especially in Colleges of Advanced Technology.

Applicants should preferably be university graduates in one of the social sciences or psychology, and have experience in research or social survey work. The research, which is financially supported by the Nuffield Foundation, will be under the general direction of Dr. T. Lupton. The appointment is for two years, to start as soon as possible.

Salary will be fixed on a scale from £1,370 to £1,550 according to qualifications and experience.

Application forms, obtainable from the Principal, College of Advanced Technology, Gosta Green, Birmingham 4, to be returned within 14 days after the appearance of the advertisement.

INSTITUTE OF DERMATOLOGY. TECHNICAL Assistant for Medical Mycology Laboratory. Post suitable for student studying by evening classes for science degree.—Applications to Secretary, Lisle Street, Leicester Square, W.C.2.

UNIVERSITY OF BRITISH COLUMBIA

Invites applications for the post of
ASSISTANT PROFESSOR OF METALLURGY

Attractive post in expanding Department for a man in the field of physical metallurgy, metal physics, materials science. Applicants should have Ph.D. or equivalent and subsequent academic, industrial, or research experience. Duties comprise undergraduate teaching, supervision of graduate work and participation in research at professional level. Opening available September 1, 1961. Salary $7,000 per year plus generous benefits.

Applications should be submitted by June 30 next, to Professor F. A. Forward, Head of the Department, University of British Columbia, Vancouver 8, B.C.

UNIVERSITY OF LIVERPOOL

Applications are invited for the post of Assistant Lecturer or Lecturer in the Department of Organic Chemistry. The initial salary will be within the range £800 to £1,350 per annum, according to age, qualifications and experience.

Applications, stating age, academic qualifications and experience, together with the names of three referees, should be received not later than April 29, 1961, by the Registrar, from whom further particulars of the conditions of appointment may be obtained. Quoting reference CV/N.

UNIVERSITY OF MANCHESTER

Applications are invited for two posts at the grade of Assistant Lecturer in the Department of Mathematics. For one of the posts qualifications are required in the field of pure mathematics and for the other in applied mathematics. Salary on a scale £800 to £950 per annum, with membership of the F.S.S.U. and children's allowance scheme.

Applications should be sent not later than April 25, 1961, to the Registrar, the University, Manchester, 13, from whom further particulars and forms of application may be obtained.

UNIVERSITY OF EDINBURGH

DEPARTMENT OF AGRICULTURE

Applications are invited for the post of Lecturer in the Department of Agriculture. Candidates should have a special interest in crop husbandry. Salary scale: £1,050 by £50 to £1,250; bar: £1,300 by £50 to £1,400 by £75 to £1,850 per annum, with placement according to qualifications and experience, and with superannuation benefit and family allowance where applicable. The successful candidate will be expected to take up duty on October 1, 1961.

Further particulars may be obtained from the undersigned, with whom applications (six copies), giving the names of two referees, should be lodged not later than April 29, 1961.

CHARLES H. STEWART,
Secretary to the University.

UNIVERSITY OF LEEDS

DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited for appointment as Lecturer or Assistant Lecturer in the Department of Organic Chemistry at a salary on the scale £1,050 by £50 to £1,400 by £75 to £1,850 (efficiency bar at £1,550) a year for a Lecturer or within the range £800 to £950 a year for an Assistant Lecturer, according to age, qualifications and experience.

Applications (three copies), stating date of birth, qualifications and experience, together with the names of three referees, should reach the Registrar, The University, Leeds, 2 (from whom further particulars may be obtained), not later than May 5, 1961.

D.S.I.R. REQUIRES ASSISTANT EXPERIMental Officer Bacteriologist or Microbiologist (woman preferred), at Torry Research Station, Aberdeen, to work on maintenance of type cultures for the National Collection of Industrial Bacteria. Qualifications: B.Sc. Pass degree, with bacteriology principal or subsidiary subject, or equivalent, generally expected. Experience of bacteriological work preferred. Age at least 21. Salary range: £598 (age 21) to £983.—Forms from Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting G.87/1A. Closing date April 22, 1961.

UNIVERSITY OF MANCHESTER

Applications are invited for the post of Assistant Lecturer in Inorganic Chemistry. Salary on a scale £800 to £950 per annum, with membership of the F.S.S.U. and children's allowance scheme.

Applications should be sent not later than April 15, 1961, to the Registrar, the University, Manchester 13, from whom further particulars and forms of application may be obtained.

UNIVERSITY OF CEYLON

CHAIR OF MECHANICAL ENGINEERING

Applications are invited for the above-mentioned Chair in the University of Ceylon. Preference will be given to experienced university teachers. The salary scale of the post is Rs.15,000 per annum, rising to Rs.21,000 per annum by four annual increments of Rs.600, and four annual increments of Rs.900. The Council may make the appointment at any point on the above scale according to qualifications and experience. Rent allowance (15 per cent of salary in the case of a married man and 7½ per cent in the case of an unmarried man, subject to a maximum of Rs.170 and Rs.85 per mensem respectively) will be paid. First-class passages for the Professor, his wife, and not more than three children will be provided. The post is not pensionable but the holder will become a contributor to the University Provident Fund contributing 5 per cent of salary and the University adding 10 per cent. The appointment will be for a period of four years in the first instance but may be renewed up to the age of 55 or 60.

Further particulars and information as to the method of application may be obtained from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close, in Ceylon and London, on April 30, 1961.

UNIVERSITY OF ABERDEEN

LAFARGE ALUMINOUS CEMENT RESEARCH FELLOWSHIP

A Junior Research Fellowship is offered in the Department of Chemistry for work on calcium aluminates and related compounds. The Fellowship is for three years and the annual stipend will be within the range £350 to £400 (tax-free). It is expected that the work should lead to the Ph.D. degree. Applicants (men or women) should have a good honours degree in chemistry, geology or physics, or equivalent qualifications such as A.R.I.C.

Applications, giving the names of three referees, should be lodged with Professor G. M. Burnett, Department of Chemistry, Old Aberdeen, not later than April 29, 1961.

WELSH COLLEGE OF ADVANCED TECHNOLOGY

DEPARTMENT OF CHEMISTRY AND BIOLOGY

Applications are invited for the post of Principal Lecturer, duties to commence on September 1, 1961. Applicants should have high academic qualifications, together with research and teaching experience. Preference will be given to an organic chemist with experience in the field of polymer chemistry or technology. An active interest in research is essential. Salary £1,750 by £50 to £1,900.

Further particulars and forms of application may be obtained on application to the Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff, to whom completed forms should be returned by April 30.

ROBERT E. PRESSWOOD,
Clerk to the Governors.

ROYAL BUCKINGHAMSHIRE AND ASSOCIATED HOSPITALS MANAGEMENT COMMITTEE

STOKE MANDEVILLE HOSPITAL, AYLESBURY, BUCKS

Applications are invited from Biochemists for the appointment of Research Biochemist (Senior Grade) in the National Spinal Injuries Centre. The successful applicant, who will be a member of the team working under the direction of the Consultant-in-charge of the Centre, should have had at least five years' experience in the basic grade of Biochemist. The post may be advanced to Principal Grade in the light of further experience and the ability of the person holding the post. The appointment will be subject to three months' notice in writing on either side and the salary will be on the scale £1,250 by £50 to £1,300 by £55 (3) to £1,465 by £60 (3) to £1,645 by £55 to £1,700 per annum.

Applications, giving details of qualifications and experience, together with names and addresses of three referees, should be sent to the Secretary, 9 Bicester Road, Aylesbury, as soon as possible.

GEOLOGICAL SURVEY AND MUSEUM (D.S.I.R.) requires an Assistant Experimental Officer for duties in Northern Ireland (based on Belfast) after preliminary training in London. Qualifications: Pass degree in geology. Salary: £645 (age 22) to £801 (age 26) to £983. Promotion prospects up to £1,336.—Forms from Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting G.99/1A. Closing date April 22, 1961.

PHARMACOLOGIST

required to work in London for the first two years in an academic department and thereafter in a newly created Research Laboratory in London.

Non-contributory Pension and Life Assurance Scheme and excellent salary; scope for promotion.

Write, giving full details of age, qualifications, experience and salary required, to the Managing Director, Biorex Laboratories Limited, 47/51 Exmouth Market, London, E.C.1.

FEDERAL GOVERNMENT OF NIGERIA

TECHNICAL OFFICER

(UTILIZATION)

Required by the Forestry Research Division of the Ministry of Economic Development in Ibadan to take charge of one or more sections of the new Forest Products Research Laboratory. These sections include Woodworking, Seasoning, Impregnation, and Timber Mechanics.

Candidates must possess a Diploma or City and Guilds Final Certificate in Machine Woodworking, Wood Technology or allied subject, and an aptitude for the careful work necessary in research. Practical experience of Machine Woodworking, Seasoning, Impregnation, or Timber Mechanics would be an advantage. Candidates should be between the ages of 22 and 50 years.

Appointment on contract for one tour of 12-18 months in the first instance with prospect of extension. Salary in the scale £936 to £1,554 per annum (including inducement addition). Gratuity of £100 to £150 per annum on satisfactory completion of service. Outfit allowance of £60 on salaries under £1,741. Free passages for officer and wife. Children's allowances while separated. Liberal home leave on full pay. Income tax at low local rate. Quarters at low rent.

Write for application forms and further particulars within 10 days of publication, stating briefly age, qualifications and experience, to the Recruitment Attache, Office of the High Commissioner for Nigeria, Nigeria House, 9 Northumberland Avenue, London, W.C.2, quoting L.2/3.

UNIVERSITY OF GLASGOW

LECTURESHIPS IN MECHANICAL ENGINEERING

Applications are invited for Lectureships in Mechanical Engineering, tenable from October 1, 1961, or such earlier date as may be agreed. Salary scale: £1,050 by £1,850 per annum. Initial salary above the minimum for those with suitable professional qualifications and experience. F.S.S.U. and family allowance benefits. Applicants should be qualified in one of the following fields: (I) Applied Mechanics, including Automatic Control. (II) Applied Thermodynamics. (III) Engineering Materials (entirely new teaching duties). New laboratories and equipment provide excellent scope for teaching and research.

Applications (eight copies), should be lodged, not later than May 5, 1961, with the undersigned, from whom further particulars may be obtained.

ROBT. T. HUTCHESON,
Secretary of University Court.

UNIVERSITY COLLEGE

ADDIS ABABA

ETHIOPIA

Lecturers in Accounting, English Language and Literature, Geography, Pharmacy, Biochemistry, Inorganic Chemistry, Botany, Mathematics, Physics, Statistics, Zoology (Comparative Anatomy). Annual net salary £1,741 to £2,500 according to qualifications and teaching experience. Accommodation and passage.

Apply to President, University College, P.O.B. 399, Addis Ababa, Ethiopia.

CUMBERLAND EDUCATION COMMITTEE

WHITEHAVEN COLLEGE OF FURTHER EDUCATION

Principal: J. Nicholson, B.Sc., A.R.I.C., A.I.M., F.C.S., F.R.S.A., Dip.Ed.

DEPARTMENT OF PHYSICS

Lecturer required for September 1, 1961, to teach Physics up to the level of the Grad.Inst.P., and to assist with Radiation and Health Physics courses. Candidates should hold a good honours degree, or an honours diploma in technology in applied physics, and have qualifications or an interest in nuclear science. Teaching, Research and/or Industrial experience an advantage. Opened in October, 1955, the College will shortly occupy considerable new extensions, including laboratories for work in the field of radioactivity. Whitehaven is situated within easy reach of the Lake District. Burnham Salary scale: £1,370 to £1,550.

Application forms (to be returned by April 24) and further particulars from the Principal, College of Further Education, Flatt Walks, Whitehaven.

GORDON S. BESSEY,
Director of Education.

5 Portland Square,
Carlisle.

UNIVERSITY COLLEGE OF NORTH WALES

BANGOR

Applications are invited for a Lectureship in the Department of Biochemistry and Soil Science. The holder of this post will be concerned in the teaching of soil science and plant nutrition: research interests in the field of metabolism of soil organic matter, or soil-plant inter-relationships, would be an advantage. Qualifications in biochemistry or chemistry are necessary, and a formal training in soils desirable. The salary scale is £1,050 by £50 to £1,400 by £75 to £1,850, and in addition there are F.S.S.U. benefits and family allowances. The appointment will date from October 1, 1961.

Two copies of the application should reach the Registrar, University College of North Wales, Bangor, from whom further particulars may be obtained, not later than April 24, 1961.

UNIVERSITY OF SHEFFIELD

POST-DOCTORAL FELLOWSHIP

Applications are invited for a Post-doctoral Fellowship (stipend £1,050 per annum or £950 plus F.S.S.U.) in the Department of Chemistry. The Fellow will be expected to carry out researches into the reactions of halogen atoms with organic molecules in collaboration with Dr. J. M. Tedder.

Applications, together with the names and addresses of three referees, should be sent to the Registrar, The University, Sheffield 10, by May 6, 1961.

UNIVERSITY COLLEGE OF NORTH STAFFORDSHIRE

Applications are invited for the post of Demonstrator in Chemistry. The appointment is tenable from September 1, 1961. Remuneration £620 per annum.

Applications should reach the Registrar, The College, Keele, Staffs, from whom further particulars and application forms may be obtained, not later than April 14, 1961.

UNIVERSITY OF MANCHESTER

Applications are invited for the post of Lecturer in Cytology in the Departments of Botany and Zoology in the Faculty of Science. Candidates should have a wide interest in the subject so as to be able to lecture to students of both departments. Duties to commence on October 1, 1961. Membership of F.S.S.U. and Children's Allowance Scheme. Salary on a scale £900 to £1,650 per annum, initial salary according to qualifications and experience.

Applications should be sent not later than April 25, 1961, to the Registrar, the University, Manchester, 13, from whom further particulars and forms of application may be obtained.

GODALMING GROUP LABORATORY

Science Graduate to carry out biochemical tests in area laboratory. Successful applicant will be graded Biochemist. Whitley Council salary scale according to qualifications and experience.

Apply to Group Pathologist, Group Laboratory, c/o St. Thomas' Hospital, Hydestile, Godalming, Surrey.

UNIVERSITY OF BRISTOL
DEPARTMENT OF MATHEMATICS

Applications are invited for the following posts tenable from August 1, 1961: Lecturer (Grade II) in Pure Mathematics. Lecturer (Grade II) in Statistics. Assistant Lecturer (Grade III) in Pure Mathematics.

Full particulars concerning these posts may be obtained from the Registrar, the University, Bristol, 8, to whom applications should be sent by May 8.

GLASSBLOWER
CHEMISTRY DEPARTMENT, QUEEN'S UNIVERSITY, KINGSTON, ONTARIO, CANADA

A Glassblower is required for the Chemistry Department. Salary according to qualifications.

Please write, including two recommendations, to Dr. J. K. N. Jones.

UNIVERSITY OF READING

Applications are invited for an Assistant Lectureship in Botany. Preference will be given to candidates with interests in ecology, particularly its physiological aspects.

Further particulars may be obtained from the Registrar (Room 22, O.R.B.), the University, Reading, by whom applications must be received not later than May 6, 1961.

DEPUTY CHIEF ANALYST. QUALIFIED chemists are invited to apply for this appointment in new City of London Research Laboratories. The work is varied but mainly concerned with food products. Experience in a Public Analyst Laboratory an advantage.—Write, stating age, qualifications and experience, to Box 961, T. G. Scott and Son, Ltd., 1 Clement's Inn, London, W.C.2.

DEPARTMENT OF AGRICULTURE AND Fisheries for Scotland, Marine Laboratory, Aberdeen. Unpensionable post for male Assistant Experimental Officer or Experimental Officer in Parasitology Unit. Qualifications should normally include good S.L.C. or G.C.E. or equivalent or university degree or H.N.C. Age 18 but under 31 on December 31, 1961. Duties include short periods at sea. Salary scales: Assistant Experimental Officer, £458 (age 18) to £801 (26 and over); scale maximum £983. Experimental Officer, £1,087 to £1,336 commencing at minimum. Opportunities to gain pensionable post.—Particulars from Establishment Officer, Room 73, Department of Agriculture and Fisheries for Scotland, St. Andrew's House, Edinburgh, 1. Closing date April 24, 1961.

LEVY WEST LABORATORIES OF Wembley require a graduate for work on very pure inorganic chemicals. Some physical leanings useful. Outside experience helpful but not essential. Salary according to qualifications.—Write or telephone Wembley 1088.

PHYSIOLOGIST, LECTURER, DEPARTment of Physiology, University of Ottawa, Ottawa, Canada. Applicants should have an M.D. or a Ph.D. degree. Salary up to $6,000, depending on experience. Pension plan, hospital insurance.—Applications and inquiries should be forwarded to Professor M. Beznak at the above address.

UNIVERSITY OF READING. APPLICAtions are invited for the newly-created Professorship of the Physical Properties of Materials, to be established in October, 1961.—Further particulars may be obtained from the Registrar (Room 22, O.R.B.), the University, Reading, by whom applications must be received not later than May 6, 1961.

MINERALOGIST, PREFERABLY WITH Ph.D. degree, salary and appointment according to training and experience. Vacancy to be filled prior to September 1, 1961.—Department of Geology, University of New Brunswick, Fredericton, New Brunswick, Canada.

QUEEN ELIZABETH COLLEGE
(UNIVERSITY OF LONDON) CAMPDEN HILL ROAD, W.8

Applications are invited for the post of Assistant Lecturer in Inorganic Chemistry vacant October, 1961.

Further particulars from the Secretary.

RESEARCH ASSISTANT WANTED AT Stoke Mandeville Hospital to help with current research on an immunological project connected with Rheumatoid Arthritis. The person appointed would work directly with the Pathologist undertaking the research. Salary £750 to £1,000 per annum according to qualifications.—Inquiries and applications, giving details of experience and the names of two referees, to the Pathologist, Stoke Mandeville Hospital, Aylesbury, Bucks.

LECTURES AND COURSES

SOUTH-WEST ESSEX TECHNICAL COLLEGE
WALTHAMSTOW, LONDON, E.17 SCIENCE DEPARTMENT

A one-day course of lectures and demonstrations on the "Theory and Practice of Glass Electrodes" will be held in the Department on April 22, 1961. Overall fee, including lunch and tea: £1 5s.

Further details and application forms may be obtained from the Head of the Science Department.

TRADE ANNOUNCEMENTS

UNWANTED MICROSCOPE — TURN IT into cash. We buy at top prices microscopes up to 50 years old, also barographs. Send or bring to the specialists. Wallace Heaton Ltd., 127 New Bond Street, London, W.1.

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Available for graduates, or candidates with equivalent qualifications. Scholarships up to £500 per annum, tenable for two years or more in the Department of Chemistry, Colour Chemistry and Dyeing and/or other Departments. Open to candidates of either sex.

Further particulars and application forms obtainable from the Principal.

H. GRAY,
Clerk to the Governors.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

A Research Studentship, value £500 per annum plus fees, is available for research on air compression. The work is expected to provide material for M.Sc. or Ph.D. thesis.

Applications from graduates or prospective graduates in physics, chemistry or engineering should be sent before May 1 to Dr. M. J. G. Wilson, Chemical Engineering Dept., Imperial College, London, S.W.7, from whom further particulars are available.

AUSTRALIAN NATIONAL UNIVERSITY
RESEARCH SCHOLARSHIPS

Applications are invited for Scholarships as follows:

(a) The John Curtin School of Medical Research. Students may enrol in the Departments of Biochemistry, Experimental Pathology, Medical Chemistry, Microbiology, Physical Biochemistry and Physiology, and in the Inorganic Chemistry Unit.

(b) The Research School of Physical Sciences. Students may enrol in the Departments of Astronomy, Geophysics, Nuclear Physics and Theoretical Physics.

(c) The Research Schools of Social Sciences and Pacific Studies. Students may enrol to undertake research in the subjects of Demography, Geography and Statistics.

(d) School of General Studies. Students may enrol to undertake research in the Departments of Botany, Chemistry, Geology, Physics and Zoology.

Applicants should be postgraduate students with capacity for research. Scholars are expected to enrol for a Ph.D. degree. Scholarships are tenable two years and may be renewed for a third year. The present value of a scholarship is £A.1,347 for medically qualified scholars. For scholars with other qualifications the basic allowance is £A.925. Married scholars with dependent children may be granted an additional allowance of £A.265 per annum in respect of the first child and a further £A.80 per annum for each other child. A grant will be made towards the cost of a scholar's fares.

Application forms and further particulars may be obtained from the University or from the Secretary, the Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications should reach the University by April 30 or October 31 in any year, although special consideration may be given to applications at other times. Scholarships may be taken up at any time after award, subject to agreement of the Head of Department concerned.

D. K. R. HODGKIN,
Registrar.

Institute of Advanced Studies,
Box 4, G.P.O.,
Canberra, A.C.T., Australia.

UNIVERSITY OF ABERDEEN
DEPARTMENT OF CHEMISTRY

Applications are invited for a Tutorial Studentship in the Department of Chemistry. The successful applicant will be required to assist in demonstrating and tutorial work in the Department. He will be expected to engage in research and will be eligible for registration as a candidate for the degree of M.Sc. or Ph.D. The stipend will be not less than £400 per annum plus fees for demonstration work.

Applications, giving the names of three referees, should be lodged with Professor G. M. Burnett, Department of Chemistry, Old Aberdeen, not later than April 29, 1961.

MISCELLANEOUS

STAFFORDSHIRE COUNTY COUNCIL EDUCATION COMMITTEE
NEW COLLEGE OF TECHNOLOGY, STAFFORD

Tenders are invited for the supply of: Major Items of Equipment for Nucleonic Laboratory (complete after-sales servicing facilities required).

Forms of Tender may be obtained from the Director of Education (Supplies), County Education Offices, Earl Street, Stafford.

Completed Tenders enclosed in the envelope provided must be received by the undersigned at County Buildings, Martin Street, Stafford, not later than noon, Saturday, May 6, 1961.

T. H. EVANS,
Clerk of the County Council.

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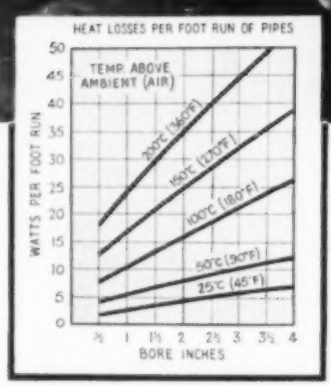

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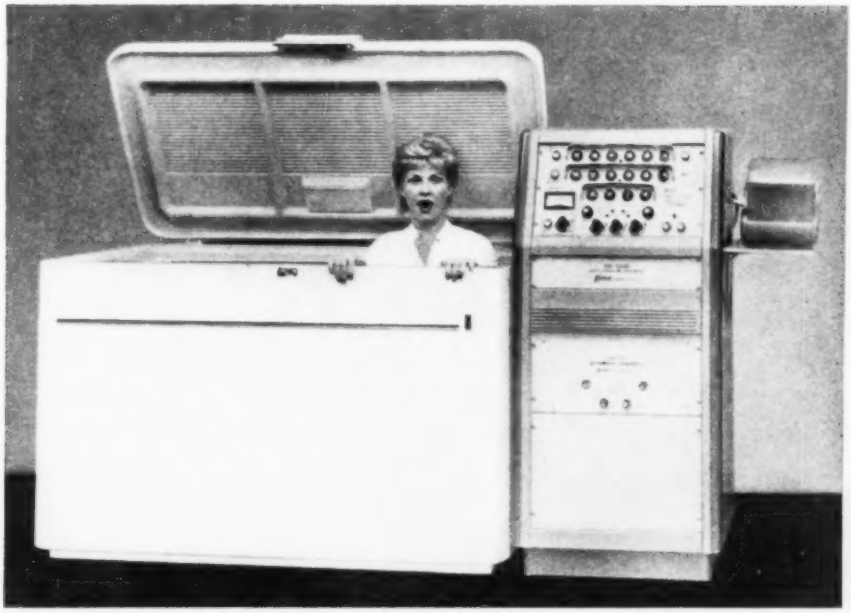

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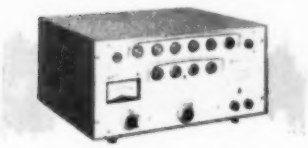

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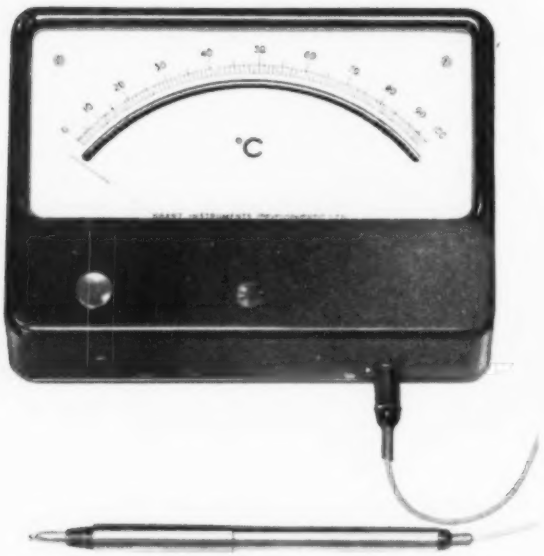

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